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13. SUPPLEMENTARY NOTES

14. ABSTRACT
The role of Project 1 in this Program Project is: 1. To preserve tissue from 72 brains, according to a standardized protocol for neuropathological studies (project # 1) and for morphometric studies (project# 2); 2. To implement clinical and neuropathological exclusion criteria to reduce the risk of distortion of results and conclusions by comorbidity, pre-, peri, and postmortem tissue changes, (3) to define type, topography and severity of qualitative developmental alterations in idiopathic autism and autism associated with dup15, and (4) to examine correlations between focal developmental changes and epilepsy and sudden death. Severe microcephaly, with brain weight reduced by 300 g is one of the most significant signs of global encephalopathy increasing the risk of epilepsy in dup 15 cohort. 2.8 times more frequent developmental alterations, especially common in the hippocampal formation of autistic subjects with dup15, and presence up to 11 different types of developmental alterations are the major contributors to early onset of epilepsy and high risk of SUDEP. Reduced volume of neurons in a majority of subcortical structures and some cortical regions in the brain of autistic children with known and unknown etiology indicates that altered trajectory of neuron growth and desynchronization of neuronal development is a common denominator for autism regardless of etiology and is linked to autistic phenotype and intellectual deficits. However, different pattern of developmental deficits neuron volume in idiopathic autism (most severe volume deficit in 4-8 years old subjects and correction of neuron size in late childhood) than in autism associated with dup(15) (permanent neuron volume deficit regardless of age) indicates that etiology defines the trajectory of neuron and brain development.

15. SUBJECT TERMS
Autism, Developmental Delay of Neuronal Growth, Desynchronization of Brain Development

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Subproject 1 (PI: Thomas Wisniewski)
Annual Report

Annual Report #4
August 28, 2012

Program Project Title: Characterization of the Pathological and Biochemical Markers that Correlate to the Clinical Features of Autism

Program Project PI: Jerzy Wegiel, Ph.D.; Co-PI: W. Ted Brown, M.D., Ph.D.

The overall aim of this multidisciplinary program project is to establish correlations between morphological and biochemical markers of autism and the clinical symptoms of the disorder.

SUBPROJECT 1

The neuropathological markers of abnormal brain development and aging in autism

Subproject 1 P.I.: Thomas Wisniewski, M.D.

INTRODUCTION
The overall aim of this multidisciplinary program project is to establish correlations between morphological and biochemical markers of autism and clinical symptoms of disease. To achieve these goals, we proposed three subprojects. The factor integrating these three closely collaborating groups is the concentration of a broad spectrum of aims and methods on brains of 72 subjects including: 32 brains of autistic people, 12 brains of individuals with autism associated with chromosome15 duplication (dup15) and 28 brains of control subjects.

This Program Project is focused on the detection of:
(a)   mechanisms leading to morphological changes and the clinical autism phenotype,
(b)   morphological and biochemical markers of autism,
(c)   correlations between pathology and clinical manifestations of autism, and
(d)   those pathological domains that might be a target for treatment.

Progress of work is consistent with the original Program Project and Project 1 aims and timetable.

Material : We examined 72 subjects including: 32 brains of autistic people, 12 brains of individuals with autism associated with chromosome15 duplication (dup15) and 28 brains of control subjects, exceeding the original plan by approximately 30%.

Project 1 plays a dual role in the Program Project and its function is reflected in the technical and research aims:

Technical aims:

1. To preserve tissue from 72 brains, according to a standardized protocol for neuropathological studies (project # 1) and for morphometric studies (project# 2).
2. To implement clinical and neuropathological exclusion criteria to reduce the risk of results and conclusions distortion by comorbidity, postmortem tissue changes, and pathology associated with mechanisms leading to death.

Tissue preservation includes: brain hemisphere fixation, dehydration, embedding, cutting, staining/immunostaining. Two standardized protocols are applied: celloidin and polyethylene glycol (PEG) embedding protocols. The celloidin protocol provides 200-um-thick sections mainly for brain neuropathology and morphometry. The PEG protocol provides 50-um-thick sections for neuropathology and immunocytochemistry based morphometry (link between biochemistry and morphology/morphometry). The common denominator of both protocols is preservation of the entire brain hemisphere for a unique power (a) extended protocol of neuropathological evaluation and (b) complex morphometric study of 17 brain regions selected to monitor potential link between structural developmental defects and three diagnostic domains of autism (social and communication deficits, and ritualistic behaviors) and intellectual deficits.

This process is monitored by a computerized system of brain tissue samples, sections and histological slides trafficking and storage. This Neuropathological Database is linked to the Project 2 Morphometric Database.

Research aims:
(a) To determine the type, topography and severity of developmental changes including defects of neurogenesis, migration and cytoarchitecture;
(b) To establish clinicopathological correlations and criteria for subclassification of the examined cohort according to clinical, neuropathological, morphometric and biochemical phenotypes. This aim is executed by cooperation with the Principal Investigators of subproject 2 and 3.
Cases not meeting the ADI-R criteria and cases with signs of comorbidity, perimortem and postmortem changes affecting brain structure were excluded from the morphometric studies.

Project #1 staff has significant contribution to Program Project publications in 2010-12.
   a. Original paper and chapter were published in 2010.
   b. Two papers were published in 2012.
   c. Two others were submitted for publication in 2012.
   d. One chapter has been submitted in 2012 and one is in preparation for publication in 2013.
   e. Two other projects are in progress.

Outcome: Historically, this is the largest postmortem morphological, morphometric, and biochemical multidisciplinary study integrating efforts of several groups concentrated on the link between etiology, genetic defects, developmental and age-associated changes of brain structure and metabolism contributing to clinical phenotype of autism (See: Key Research Accomplishments and Conclusions).

Request for no-cost extension. To complete studies in progress and to respond to reviewers' requests, we are asking for a no-cost extension of Project 1 and the other two Projects (2-Dr. Jerzy Wegiel and 3 – Dr Abha Chauhan).

BODY

1.   Differences Between the Pattern of Developmental Abnormalities in Autism Associated with Duplications 15q11.2-q13 and Idiopathic Autism
Wegiel J, Schanen CN, Cook EH, Sigman M, Brown WT, Kuchna I, Nowicki K, Wegiel J, Imaki H, Ma SY, Marchi E, Wierzba-Bobrowicz T, Chauhan A, Chauhan V, Cohen IL, London E, Flory M, Lach B, Wisniewski T. J Neuropathol Exp Neurol 2012, 71, 382-397.

Approximately 5%–10% of individuals with an ASD have an identifiable genetic etiology corresponding to a known single gene disorder, such as fragile X syndrome, or chromosomal rearrangements, including maternal duplication of 15q11-q13. The symptoms in people with idic(15) markers are correlated with the extent of duplication of the Prader Willi syndrome/Angelman syndrome critical region (PWS/ASCR) (15q11-q13) (Cheng et al 1994, Robinson et al 1993). Larger supernumerary idic(15) chromosomes, are associated with a cluster of clinical features that include autistic behavior, intellectual deficit (ID), seizures, hypotonia, hyperactivity, and irritability (Wisniewski et al 1979). Duplications of chromosome 15q11-q13 account for approximately 0.5%–3% of ASD and may be the most prevalent cytogenetic aberration associated with autism in most studies (Cook et al 1997, Schroer et al 1998, Gillberg et al 1991, Ghaziuddin et al 1993, Baker et al 1994, Bunday et al 1994).

We hypothesized that neuropathology of autism with dup(15) differs from that of idiopathic autism and provides an explanation for the high prevalence of seizures and associated sudden death in dup(15) cohort. The purpose of this study was to detect the difference between the patterns of developmental abnormalities in the brains in autism of unknown etiology (idiopathic autism) and individuals with duplications of chromosome 15q11.2-q13 [dup(15)] and autism, and to identify alterations that may contribute to seizures and sudden death in dup(15) syndrome. Brains of the 9 subjects with dup(15), 10 with idiopathic autism and 7 control subjects were examined. In dup(15) cohort seven subjects (78%) were diagnosed with autism, 7 had seizures (78%), and in 6 cases (67%) sudden unexplained death was reported. Subjects diagnosed with dup15 autism were microcephalic, with the mean brain weight 300g less (1,177 g) than in idiopathic autism (1,477 g; p<0.001). Heterotopias in the alveus, CA4 and dentate gyrus, and dentate gyrus dysplasia were detected in 89% of subjects with dup(15) autism but in only 10% of subjects with idiopathic autism (p<0.001). However cerebral cortex dysplasia was detected in 50% of subjects with idiopathic autism and in none of dup(15) autism cases (p<0.04). Different spectrum and higher prevalence of developmental neuropathological changes than those seen in idiopathic autism may contribute to high risk of early onset of seizures and sudden death in the dup(15) cohort.

2.   Clinicopathological Stratification of Idiopathic Autism and Autism Associated with Duplications 15q11.2-q13
Wegiel J, Schanen NC, Cook EH, Brown WT, Kuchna I, Nowicki K, Wegiel J, Imaki H, Ma SY, London E, Wisniewski T. Chapter in press: The Neuroscience of Autism Spectrum Disorders; Editors: Joseph Buxbaum and Patrick Hof, Elsevier Inc. 2013

Postmortem studies of brains of individuals with idiopathic autism and duplications 15q11.2-q13 autism identify a cluster of neuropathological features differentiating these cohorts. They show a need for both sub-classification of autism according to etiology, clinical

presentation, and neuropathology, and a commonality of clinical and neuropathological traits justifying autism diagnosis. The features differentiating these cohorts include: (a) maternal origin dup(15), (b) autism in 78% of subjects, (c) more severe clinical phenotypes, with intellectual deficit (100%), early-onset of severe or intractable seizures in 78% of subjects, and increased to 67% prevalence of sudden unexplained death, (d) high prevalence of microcephaly, with mean brain weight 300g less than in idiopathic autism, (e) several-fold increase in the number of developmental abnormalities, including defects of migration and dysplastic changes, especially numerous in the hippocampal formation, and (f) significant increase of the intraneuronal amyloid load, reflecting enhanced amyloid-β precursor protein processing with α-secretase.

KEY RESEARCH ACCOMPLISHMENTS

Project 1 has a significant contribution to three major research strategies of the Program Project, including the study of the contribution of:
1. Qualitative developmental abnormalities to the autistic phenotype.
2. Quantitative developmental abnormalities to the autistic phenotype.
3. Developmental neuronal metabolic alterations to the clinical phenotype of autism

Major accomplishments:
1. Thanks to DOD grant and Autism Speaks and Autism Tissue Program support in tissue acquisition in past four years, we were able to preserve historically the largest collection of unique quality brain tissue samples (72 brain hemispheres) including:
   - 32 brain hemispheres of people with idiopathic autism,
   - 12 brains hemispheres of people with dup15 autism,
   - 28 control brain hemispheres.
2. Brain hemispheres cut into serial sections provided material for several research strategies. Therefore we were able to expand spectrum of research targets and hypotheses tested in this Program Project.
3. Neuropathological component (Project #1) provided neuropathological reports and exclusion criteria reducing risk of distortion of research results by comorbidity, pre-, peri- and postmortem changes.
4. The study determined the contribution of qualitative developmental abnormalities to the autistic phenotype in autism with an unknown etiology and autism caused by maternal origin dup(15) (Wegiel et al 2010a and b Wegiel et al 2012).
5. We identified both, (a) differences between the pattern of developmental abnormalities in autism associated with duplications 15q11.2-q13 and autism of unknown origin and (b) core neuropathology present in autism regardless of autism etiology.
6. Project results in detection of focal abnormalities that play a key role in early onset of epilepsy, functional regression and an increased risk of Sudden Unexpected Death in Epilepsy (SUDEP).

REPORTABLE OUTCOMES

1.   Wegiel J, Wisniewski T, Chauhan A, Chauhan V, Kuchna I, Nowicki K, Imaki H, Wegiel J, Ma SY, Wierzba-Bobrowicz T, Cohen IL, London E, Brown WT. Type, topography and sequelae of neuropathological changes shaping clinical phenotype of autism. In: Autism: Oxidative Stress, Inflammation, and Immune Abnormalities. Ed.: Abha Chauhan, Ved Chauhan and W. Ted Brown. Taylor & Francis/CRC Press, Boca Raton, FL, 2010, pp. 1-34.

2.   Wegiel J, Kuchna I, Nowicki K, Imaki H, Wegiel J, Marchi E, Ma SY, Chauhan A, Chauhan V, Wierzba Bobrowicz T, de Leon M, Saint Louis LA, Cohen IL, London E, Brown WT, Wisniewski T. The neuropathology of autism: defects of neurogenesis and neuronal migration, and dysplastic changes. Acta Neuropathologica, 2010, 119,755-770.

3.   Wegiel J, Schanen CN, Cook EH, Sigman M, Brown WT, Kuchna I, Nowicki K, Wegiel J, Imaki H, Ma SY, Marchi E, Wierzba-Bobrowicz T, Chauhan A, Chauhan V, Cohen IL, London E, Flory M, Lach B, Wisniewski T. Differences Between the Pattern of Developmental Abnormalities in Autism Associated with Duplications 15q11.2-q13 and Idiopathic Autism. J Neuropathol Exp Neurol 2012, 71, 382-397.

4.   Wegiel J, Frackowiak J, Mazur Kolecka B, Schanen NC, Cook EH, Sigman M, Brown WT, Kuchna I, Wegiel J, Nowicki K, Imaki H, Ma SY, Chauhan A, Chauhan V, Miller DL, Mehta PD, Cohen IL, London E, Reisberg B, de Leon MJ, Wisniewski T. Amyloid Abnormal Intracellular Accumulation and Extracellular Aβ Deposition in Idiopathic and Dup15q11.2-q13 Autism Spectrum Disorders. PloS ONE 2012, 7, e35414.

5.   Wegiel J, Kuchna I, Nowicki K, Imaki H, Wegiel J, Ma SY, Azmitia EC, Banerjee P, Chauhan A, Chauhan W, Cohen IL, London E, Brown WT, Wisniewski T. Contribution of Olivo-floccular Circuitry Developmental Defects to Atypical Gaze in Autism. Submitted to J Autism and Dev Disabilities.

6.   Wegiel J, Schanen NC, Cook EH, Brown WT, Kuchna I, Nowicki K, Wegiel J, Imaki H, Ma SY, London E, Wisniewski T. Clinicopathological Stratification of Idiopathic Autism and Autism Associated with Duplications 15q11.2-q13. Chapter in press: The Neuroscience of Autism Spectrum Disorders; Editors: Joseph Buxbaum and Patrick Hof, Elsevier Inc. 2013

7.   Wegiel J, Flory M, Kuchna I, Nowicki K, Ma SY, Imaki H, Wegiel J, Cohen IL, London E, Brown WT, Wisniewski T. Brain region– and neuron-type–specific delay of neuronal growth desynchronizes brain development and maturation in autism. Submitted to Acta Neuropathologica.

8.   Wegiel J, Morys J, Ma SY, Kuchna I, Nowicki K, Imaki H, Wegiel J, Flory M, Brown WT, Wisniewski T. Delayed development of claustrum in autism. Chapter in preparation for submission: Functional Neuroanatomy of the Claustrum. Edited by: John Smythies, Lawrence Edelstein, V.S. Ramachandran, Elsevier 2013

9.   Wegiel J, Flory M, Kuchna I, Nowicki K, Ma SM, Imaki H, Wegiel J, Cohen IL, London E, Brown WT, Wisniewski T. Different trajectories of abnormal neuronal growth desynchronize brain development in autism associated with dup15 and autism of unknown origin. Project in progress.

10.   Frackowiak J, Mazur-Kolecka B, Kuchna I, Brown WT, Wegiel J. Amyloid-beta and lipid oxidation in the brain cortex in autism. Project in progress. Poster presented at the Cell Development meeting at Santa Cruz, August 2012. Paper in preparation for submission.

11.   Wegiel J, Lightfoot D, Pickett J, Brown WT. New trends in brain tissue banking for autism research. Autism Spectrum News 2012, 4, no3.

Meetings (2012)

1.   Wegiel J, Schanen NC, Cook EH, Brown WT, Kuchna I, Nowicki K, Wegiel J, Imaki H, Ma SY, London E, Wisniewski T. Results of application of new methods of tissue handling, distribution, and sharing for research on autism. Contributions of postmortem tissue to the study of Developmental Disorders. 20th Anniversary of the NICHD Brain and Tissue Bank for Developmental Disorders. Bethesda, July 16-17, 2012

2.   Wegiel J, Schanen NC, Cook EH, Brown WT, Kuchna I, Nowicki K, Wegiel J, Imaki H, Ma SY, London E, Wisniewski T. Clinicopathological stratification of idiopathic autism and autism associated with duplications 15q11.2-q13. Dup15q Alliance 2012 Scientific Meeting. Boston Children's Hospital, Boston August 9-10, 2012

3.   Frackowiak J, Mazur-Kolecka B, Kuchna I, Brown WT, Wegiel J. Amyloid-beta and lipid oxidation in the brain cortex in autism. Cell Development Meeting at Santa Cruz, August 2012.

CONCLUSIONS

Focal dysplasia and heterotopias are the major cause of increased risk of sudden unexpected death in early childhood in autism associated with dup15

1. Severe microcephaly, with brain weight reduced by 300 g is one of the most significant signs of global encephalopathy increasing the risk of epilepsy.
2. 2.8 times more frequent developmental alterations, especially common in the hippocamapl formation of autistic subjects with dup15, and presence up to 11 different types of developmental alterations are the major contributor to early onset of epilepsy and high risk of SUDEP.
2. Reduced volume of neurons in a majority of subcortical structures and some cortical regions in the brain of autistic children 4–8 years of age appear to reflect brain immaturity in early childhood contributing to autism and intellectual deficit.
3. Combination of all of these developmental defects increases risk of death at a very early age (~10 years) in autism associated with dup15.

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APPENDICES

1.   Wegiel J, Schanen CN, Cook EH, Sigman M, Brown WT, Kuchna I, Nowicki K, Wegiel J, Imaki H, Ma SY, Marchi E, Wierzba-Bobrowicz T, Chauhan A, Chauhan V, Cohen IL, London E, Flory M, Lach B, Wisniewski T. Differences Between the Pattern of Developmental Abnormalities in Autism Associated with Duplications 15q11.2-q13 and Idiopathic Autism. J Neuropathol Exp Neurol 2012, 71, 382-397.

2.   Wegiel J, Schanen NC, Cook EH, Brown WT, Kuchna I, Nowicki K, Wegiel J, Imaki H, Ma SY, London E, Wisniewski T. Clinicopathological Stratification of Idiopathic Autism and Autism Associated with Duplications 15q11.2-q13. Chapter in press: The Neuroscience of Autism Spectrum Disorders; Editors: Joseph Buxbaum and Patrick Hof, Elsevier Inc. 2013

ORIGINAL ARTICLE

Differences Between the Pattern of Developmental Abnormalities in Autism Associated With Duplications 15q11.2-q13 and Idiopathic Autism

Jerzy Wegiel, PhD, N. Carolyn Schanen, MD, PhD, Edwin H. Cook, MD, Marian Sigman, MD,
W. Ted Brown, MD, PhD, Izabela Kuchna, MD, PhD, Krzysztof Nowicki, MD, Jarek Wegiel, MSc,
Humi Imaki, PhD, Shuang Yong Ma, MD, PhD, Elaine Marchi, MSc, Teresa Wierzba-Bobrowicz, MD, PhD,
Abha Chauhan, PhD, Ved Chauhan, PhD, Ira L. Cohen, PhD, Eric London, MD, Michael Flory, PhD,
Boleslaw Lach, MD, PhD, and Thomas Wisniewski, MD

Abstract

The purposes of this study were to identify differences in patterns of developmental abnormalities between the brains of individuals with autism of unknown etiology and those of individuals with duplications of chromosome 15q11.2-q13 (dup[15]) and autism and to identify alterations that may contribute to seizures and sudden death in the latter. Brains of 9 subjects with dup(15), 10 with idiopathic autism, and 7 controls were examined. In the dup(15) cohort,

7 subjects (78%) had autism, 7 (78%) had seizures, and 6 (67%) had experienced sudden unexplained death. Subjects with dup(15) autism were microcephalic, with mean brain weights 300 g less (1,177 g) than those of subjects with idiopathic autism (1,477 g; p < 0.001). Heterotopias in the alveus, CA4, and dentate gyrus and dysplasia in the dentate gyrus were detected in 89% of dup(15) autism cases but in only 10% of idiopathic autism cases (p < 0.001). By contrast, cerebral cortex dysplasia was detected in 50% of subjects with idiopathic autism and in no dup(15) autism cases (p < 0.04). The different spectrum and higher prevalence of developmental neuropathologic findings in the dup(15) cohort than in cases with idiopathic autism may contribute to the high risk of early onset of seizures and sudden death.

Key Words: Autism, Chromosome 15q11.2-q13 duplication, Developmental brain alterations, Seizures, Sudden unexpected death.

INTRODUCTION

Autism is the most severe form of autism spectrum disorder (ASD) and is characterized by qualitative impairments in reciprocal social interactions, qualitative impairments in verbal and nonverbal communication, restricted repetitive and stereotyped patterns of behavior, interests and activities, and onset before the age of 3 years (1). Autism is heterogeneous, both phenotypically and etiologically. In 44.6% of affected children, autism is associated with cognitive impairment as defined by intelligence quotient scores of less than 70 (2). Epilepsy is a comorbid complication diagnosed in up to 30% of individuals with autism (3). In 90% to 95% of cases, the etiology of autism is not known (idiopathic or nonsyndromic autism) (4, 5). Twin and family studies have indicated both a strong and a moderate heritability for ASD (6–8).

Approximately 5% to 10% of individuals with an ASD have an identifiable genetic etiology corresponding to a known single gene disorder, for example, fragile X syndrome or chromosomal rearrangements, including maternal duplication of 15q11-q13. Supernumerary isodicentric chromosome 15 [idic(15)] (formerly designated as inverted duplication 15)

is a relatively common genetic anomaly that most often leads to tetrasomy or mixed trisomy/tetrasomy of the involved segments and arises from a U-type crossover between a series of low copy repeats (LCRs) located on the proximal long arm. Small heterochromatic idic(15) chromosomes, which do not include the 15q11-q13 region, are often familial and are not associated with a clinical abnormality (9, 10). The symptoms in people with idic(15) markers correlate with the extent of duplication of the Prader-Willi syndrome/Angelman syndrome critical region (15q11-q13) (10, 11). Larger supernumerary idic(15) chromosomes, which include the imprinted chromosome 15q11-q13 region, are associated with a cluster of clinical features that include intellectual deficits (IDs), seizures, autistic behavior, hypotonia, hyperactivity, and irritability (12). Duplications of chromosome 15q11-q13 account for approximately 0.5% to 3% of ASD and may be the most prevalent cytogenetic aberration associated with autism in most studies. These duplications range from 4 to 12 Mb and may occur either through generation of supernumerary idic(15) chromosomes or as interstitial duplications and triplications. For interstitial duplications, maternal origin confers a higher risk for an abnormal phenotype (13, 14), and most of the reported chromosome 15 duplications (dup[15])

are maternally derived. A small number of subjects with duplications of paternal origin have been variously reported as being unaffected (13, 15–17), affected but without ASD (16, 18), or affected with ASD (19). Interstitial triplications (int trp[15]) are relatively rare but have invariably been associated with a severe phenotype, including ID, ASD, or autistic features, and frequently with seizures. The parent-of-origin effect is not evident in the reported cases of int trp(15), with both maternal and paternal triplications associated with poor outcome.

Clinical studies indicate that most individuals diagnosed with dup(15) of maternal origin fulfill the criteria for the diagnosis of autism. In the first clinical reports, 24 individuals with idic(15) and autism were identified using standardized criteria of the *Diagnostic and Statistical Manual of Mental Disorders, Third Edition, Revised* (13, 15, 20–24). In several other studies of subjects with idic(15) chromosomes, autism was clinically diagnosed, although without the application of standardized measures of autism (25–27). Application of the Gilliam Autism Rating Scale (28) to another idic(15) cohort confirmed a high prevalence of autism; 20 (69%) of 29 children and young adults with idic(15) had an ASD (29).

TABLE 1. Material Examined

Group	Case No.	Sex	Age, y	Cause of Death	PMI, h	Hem	Brain Weight, g
dup(15) autism	1	M	9	SUDEP	13.6	R	1,130
	2	M	10	SUDEP	17.7	R	1,070
	3	M	11	SUDEP	10.5	R	1,540
	4	F	15	SUDEP (suspected)	24.0	LR	1,141
	5	F	15	Pneumonia	—	LR	1,125
	6	M	20	Cardiopulmonary arrest (choking on food)	28.1	L	1,190
	7	M	24	Pneumonia	36.3	L	1,200
	8	F	26	SUDEP (suspected)	28.6	R	1,310
	9	F	39	SUDEP	32.8	R	890
			Mean (SD)		23.9 (9.2)		1,177 (177)
Idiopathic autism	1	M	2	Asphyxia (drowning)	4.0	R	1,328
	2	F	5	Asphyxia (drowning)	33.0	R	1,360
	3	M	5	Asphyxia (drowning)	25.5	R	1,560
	4	M	8	Asthma attack	13.8	R	1,740
	5	M	9	SUDC	3.7	R	1,690
	6	M	11	Asphyxia (drowning)	—	R	1,400
	7	M	28	Seizure-related	43.0	R	1,580
	8	M	32	Brain tumor (glioblastoma multiforme)	—	L	1,260
	9	M	51	Heart failure	22.2	R	1,530
	10	M	52	Heart failure	11.5	L	1,324
			Mean (SD)		19.6 (14.0)		1,477 (166)
Control	1	F	8	Rejection of cardiac transplant	20.0	R	1,340
	2	M	14	Asphyxia (hanging)	5.0	R	1,420
	3	M	14	Multiple traumatic injuries	16.0	R	1,340
	4	M	32	Heart failure	14.0	R	1,401
	5	F	33	Bronchopneumonia	6.0	L	1,260
	6	F	43	Sepsis	10.1	L	1,350
	7	M	47	Myocardial infarct	23.0	L	1,450
			Mean (SD)		13.4 (6.8)		1,366 (63)

PMI, postmortem interval; Hem, hemisphere; R, right; L, left; SUDEP, sudden unexpected death of subject with known epilepsy: SUDC, sudden unexpected death in childhood.

TABLE 2. Chromosome 15 Abnormalities in the dup(15) Cohort

Case #	Chromosomal Alterations	Prader-Willi Syndrome/Angelman Syndrome Critical Region (PWS/ASCR)	Parental Origin of Abnormality
1	47,XY,+idic(15)(q13;q13); Idic(15) arising from BP3:BP3 exchange. Subject 02-18, Wang et al (47)	Tetrasomy	Maternal
2	47,XY,+idic(15)(q13;q13); Idic(15) arising from BP4:BP5 exchange. Subject 01-22, Wang et al (47)	Tetrasomy	Maternal
3	47,XY,+der(15)(pter>q13::q13>cen>q13::q13>pter). Tricentric chromosome 15 arising from BP3:BP3 exchanges. Case 2, Mann et al (46); Subject 00-29, Wang et al (47)	Hexasomy	Maternal
4	47,XX,+idic(15)(q13;q13); Idic(15) arising from BP4:BP5 exchange. Subject 99-93, Wang et al (47)	Tetrasomy	Maternal
5	47,XX,+der(15)(q13;q13); Idic(15) arising from BP4:BP5 exchange	Tetrasomy	Maternal
6	IDIC15 (2 extra copies of region from beginning of array to BP4)	Tetrasomy	Not determined
7	47,XY,del(15)(q11.2)+idic(15)(q13;q13); Idic(15) arising from BP4:BP5 exchange; deletion of BP1:BP2 on 1 homolog of chromosome 15. Subject 00-03, Wang et al (47)	Tetrasomy	Maternal
8	47,XX,+idic(15)(q13;q13); Idic(15) arising from BP4:BP5 exchange. Subject 99-27, Wang et al (47)	Tetrasomy	Maternal
9	46,XX,trp(15)(q11.2q13). Subject 02-9, Wang et al (47)	Tetrasomy	Maternal

PWS/ASCR, Prader-Willi syndrome/Angelman syndrome critical region.

The link between the extent of genetic duplication and clinical phenotype has not yet been determined, but genomic and functional profiling provides insights into the direct and indirect effects of the copy number gains associated with chromosome 15 duplications. The γ-aminobutyric acid type A ($GABA_A$) receptor subunit genes ($\alpha5$, β, and $\gamma3$) that have been implicated in the etiology of autism (30) are located in the susceptibility segment of duplicated chromosome 15

TABLE 3. Autism Diagnostic Interview-Revised–Based Diagnosis of Autism

Group	Case No.	Reciprocal Social Interactions (10)	Communication Verbal (8)	Communication Nonverbal (7)	Restricted, Repetitive, and Stereotyped Behavior (3)	Alterations Evident Before 36 mo (1)	Diagnosis (Test)
dup(15) autism	1	7	NA	0	0	5	Autism (ADOS-G)
	2	24	NA	11	5	3	Autism (ADI-R)
	3	18	NA	2	2	5	PDD-NOS
	4	26	22	NA	12	5	Autism (ADI-R)
	5	—	—	—	—	—	Autism (ADI-R) (score not available)
	6	27	18	NA	4	5	Autism (ADI-R)
	7	23	22	NA	9	3	Autism (ADI-R)
	8	28	16	NA	9	5	Autism (ADI-R)
	9						Unknown
Idiopathic autism	1	14	NA	9	6	5	Autism (ADI-R)
	2						Autism (ADOS)
	3	22	NA	14	6	5	Autism (ADI-R)
	4	11	NA	8	2	4	Atypical autism – ASD (ADI-R)
	5	26	NA	12	5	4	Autism (ADI-R)
	6	25	20	NA	4	4	Autism (ADI-R)
	7	22	16	NA	5	3	Autism (ADI-R)
	8	—	—	—	—	—	Autism (ADI-R) (score not available)
	9	27	19	9	6	5	Autism (ADI-R)
	10	—	—	—	—	—	Atypical autism – ASD (ADI-R score not available)

ADI-R, Autism Diagnostic Interview – Revised (cutoff scores); ADOS-G, Autism Diagnostic Observation Scale – Generic (42); NA, not applicable; PDD-NOS, Pervasive Developmental Disorder – Not Otherwise Specified.

TABLE 4. Behavioral, Neurologic, and Other Clinical Observations

Group	Case No.	Psychiatric Disorders and Neurologic Symptoms	Cognitive Assessment	Seizures (Age at Onset)
dup(15) autism	1	Severe hypotonia. Regression in infancy. Abnormal response to pain and heat.	Profound ID (DQ < 20)	Infantile spasms. Intractable epilepsy (10 mo)
	2	Hypotonia. Severe regression at age of 15 mo. Head banging.	Profound ID (DQ = 22)	Intractable epilepsy (8 mo)
	3	Regression with infantile spasms. Severe hypotonia.	Profound ID (DQ < 20)	Infantile spasms. Intractable epilepsy (10 mo). Vagus nerve stimulator.
	4	Delay of motor skills. Mild to moderate spastic quadriparesis. Abnormal response to pain, cold.	Severe ID (DQ = 31)	Seizures (11 y)
	5	Hyperactive, verbal	(—)	No record
	6	Sleep disorder. Abnormal response to pain, heat and cold.	(—)	No record
	7	Abnormal gait	Profound ID (DQ < 20)	Intractable epilepsy (7 y). Vagus nerve stimulator. Callosotomy.
	8	Obsessive compulsive symptoms	Moderate ID (IQ = 36)	Epilepsy (16 y)
	9	Cerebral palsy. Microcephaly	Severe ID.	Intractable epilepsy (9 y). Vagus nerve stimulator.
		Aggressive behavior. Trichotillomania.	(—)	
Idiopathic autism	1	Self-stimulatory behavior	(—)	No record
	2	Hyperactivity, attention deficit. Sleep disorder. Enhanced sensitivity to light and sound.	Cognitive delay (IQ = 65)	No record
	3	Sleep disorder	(—)	No record
	4	Self-stimulatory behavior	(—)	Epilepsy (8 y)
	5	Hypotonia	Moderate ID	No record
	6	No record	Moderate ID	Epilepsy
	7	Bipolar disorder	(—)	Epilepsy
	8	No record	(—)	No record
	9	Enhanced sensitivity to sound, heat, and cold. Low pain threshold.	(—)	One grand malseizure
	10	Bipolar disorder, social anxiety	(—)	No record

DQ, developmental quotients; ID, intellectual deficit; —, no formal assessment of ID available.

(31–34). A map of parent-of-origin-specific epigenetic modifications suggests that this imprinted locus may have links not only with autism but also with other psychiatric phenotypes (35). Differential methylations in the 15q11-q13 region, including the $GABA_A$ gene (30, 36–38), may contribute to epigenetic modifications and a broader clinical phenotypes in dup(15)/autism. Several other genes located in or near the 15q11-q13 region may contribute to a variable phenotype of autism, including a gene for juvenile epilepsy located near D15S165 (39) and a locus for agenesis of the corpus callosum (40).

We hypothesized that the neuropathology of autism with dup(15) differs from that of idiopathic autism and that it would provide an explanation for the high prevalence of seizures and associated sudden death in the dup(15) cohort. The aim of this comparative postmortem study of the brains of individuals diagnosed with idic(15) or int trp(15) (collectively referred to as dup[15]) and of individuals with idiopathic autism was to identify common neuropathologic developmental defects for both cohorts and the patterns of changes distinguishing dup(15) from idiopathic autism. The dup(15) cohort examined exhibited a strikingly high prevalence of epilepsy, including intractable epilepsy, and a high rate of sudden unexpected death in childhood and early adult-

hood. Therefore, the second aim of the study was to identify patterns of neuropathologic changes that may contribute to epilepsy and sudden death in the dup(15) cohort.

MATERIALS AND METHODS

The cohort of subjects diagnosed with dup(15) consisted of 9 subjects (range, 9–39 years), including 5 males (55%) and 4 females (45%). The cohort with autism consisted of 10 subjects (range, 2–52 years), including 9 males (90%) and 1 female (10%). The control cohort consisted of 7 subjects from 8 to 47 years, including 4 males (43%) and 3 females (57%). The brain of 1 subject diagnosed with dup(15) was excluded because of very severe autolytic changes, and the brain of 1 control subject was excluded because of lack of information about cause of death. The mean postmortem interval varied from 23.9 hours in the dup(15) cohort, to 19.6 hours in the idiopathic autism cohort, and 13.4 hours in the control group (Table 1).

Clinical and Genetic Characteristics

Psychological, behavioral, neurologic, and psychiatric evaluation reports and reports by medical examiners and pathologists were the source of the medical records of the examined postmortem subjects. Medical records were obtained after consent for release of information from the subjects' parents. In

TABLE 5. Topography and Type of Major Developmental Alterations

		Hippocampus		Cerebral cortex	Cerebellum	
Group	Case No.	Heterotopia (Alveus, CA4, DG)	Dysplasia (DG)	Dysplasia	Heterotopia	Dysplasia
dup(15) autism	1	+	++		+	
	2	+	++			+
	3	+	++		+	+
	4	+				+
	5	+	+++		++	
	6	+	++			+
	7	+	+++++		++	+
	8		+++		+	+
	9	++	+++			—
		8 (89%)	8 (89%)	0	5 (56%)	6/8 (75%)
Idiopathic autism	1		+	+	+	+
	2			+		+
	3			+		+
	4	+		+	+	+
	5				+	
	6				+	—
	7			+	+	
	8				+	
	9					—
	10					
		1 (10%)	1 (10%)	5 (50%)	6 (60%)	4/8 (50%)
Control	1					
	2					
	3					
	4					
	5					
	6					
	7					+
		0	0	0	0	1 (14%)
p						
dup(15) vs idiopathic autism		0.001	0.001	0.03	ns	ns
dup(15) vs control		0.001	0.001	ns	0.03	0.04
Autism vs control		ns	ns	0.04	0.03	ns

+, The number of types of developmental alterations and percentages of subjects with developmental defects are in parentheses; ns, not significant; —, missing structure; DG, dentate gyrus.

Statistical analyses: Fisher exact test and Mann-Whitney U test.

most subjects diagnosed as being affected by dup(15) and idiopathic autism, the Autism Diagnostic Interview – Revised (ADI-R) was administered to the donor family after the subject's death as a standardized assessment tool to confirm an autism diagnosis (41). In addition, 6 of the subjects with dup(15) chromosomes were enrolled in a study of molecular contributors to the phenotype. The study was approved by the institutional review boards of the University of California, Los Angeles, and Nemours Biomedical Research. Before their deaths, 6 subjects (Cases 1–4, 7, and 8) had undergone behavioral and cognitive testing using the ADI-R, the Autism Diagnostic Observation Scale – Generic (ADOS-G) (42), and the Mullen Scales of Early Learning (43, 44) or the Stanford-Binet Intelligence Scales (45). Age at evaluation ranged from 45 to 251 months.

Molecular genetic evaluation using antemortem peripheral blood samples and lymphoblast cell lines for 8 of the dup(15) cases included genotyping with 19 to 33 short tandem repeat polymorphisms from chromosome 15, Southern blot analysis of dosage with 5 to 12 probes, and measurement of the methylation state at *SNRPN* exon α, as described (46). In addition, array comparative genomic hybridization was performed, using a custom bacterial artificial chromosome array (47). Morphology of the duplication was confirmed by fluorescent in situ hybridization using 5 to 8 probes that detect sequences on chromosome 15q11-q14 (46).

Tissue Preservation for Neuropathologic Study

One brain hemisphere from each subject was fixed in 10% buffered formalin, dehydrated in a graded series of ethanol, infiltrated with polyethylene glycol 400 (no. 807 485; Merck, Whitehouse Station, NJ) and embedded in fresh polyethylene glycol 1000 (48) and stored at 4°C. Tissue blocks were then cut at a temperature of 18°C into 50-μm-thick

serial sections identified with a number and stored in 70% ethyl alcohol at room temperature. Free-floating hemispheric sections were stained with cresyl violet and mounted with Acrytol. To increase the probability of detection of small focal developmental defects, on average, 140 hemispheric cresyl violet–stained sections at a distance of 1.2 mm were examined per case.

Tissue acquisition for this project was based on individual tissue transfer agreements between the project's principal investigator and several tissue banks, including (i) the National Institute of Child Health and Human Development Brain and Tissue Bank for Developmental Disorders at the University of Maryland School of Medicine, Baltimore, MD; (ii) the Harvard Brain Tissue Resource Center, McLean Hospital, Belmont, MA; and (iii) the Brain and Tissue Bank for Developmental Disabilities and Aging of the New York State Institute for Basic Research in Developmental Disabilities. The institutional review board of the Institute for Basic Research in Developmental Disabilities approved the methods applied in this study.

Statistical Analysis

Differences among groups in the presence or absence of heterotopias and dysplasias were examined using the Fisher exact test. Differences in the numbers of abnormalities were examined using the Mann-Whitney U (Wilcoxon signed ranks) test or (for comparisons of all 3 groups) the Kruskal-Wallis one-way analysis of variance (an extension of the U test), with exact probabilities computed using version 12 of the SPSS statistical package (SPSS for Windows, Release 12.0, 2003; SPSS, Inc, Chicago, IL). Brain weights were compared in one-way analysis of variances controlled for age.

RESULTS

Genetic Characteristics

For 8 subjects (Cases 1–5 and 7–9) in the dup(15) cohort, duplication chromosomes was characterized using a combination of genotyping, fluorescent in situ hybridization, Southern blot, and array comparative genomic hybridization with lymphoblasts generated from antemortem blood samples (Table 2). All were maternally derived; 7 of these subjects were tetrasomic for the imprinted region between brake points (BP)2 and BP3, although the BP involved was variable. The idic(15) present in cells from Case 1 was generated by an exchange between copies of LCR3, causing tetrasomy that extended only to BP3. Four subjects (Cases 2, 4, 5, and 8) had the most common form of idic(15) chromosomes arising by nonallelic homologous recombination (NAHR) between BP4 and BP5, leading to tetrasomy of the region between the p-arm and BP4, with trisomy for the interval between BP4 and BP5. Another subject (Case 7) had a similar idic(15) chromosome but also carried a deletion between BP1 and BP2 on 1 homolog of chromosome 15 (Subject 00-03) (47). Case 3 had a complex tricentric supernumerary chromosome arising from NAHR between multiple copies of BP3, rendering him hexasomic for the region between the centromere and BP3 (46). One subject (Case 9) had an int trp(15) chromosome that led to tetrasomy between BP1 and BP4 and trisomy for the interval between

the fourth and fifth LCRs, similar to the dosage seen in the BP4:BP5 idic(15) chromosomes.

The study of 5 subjects with idiopathic autism (Cases 1–4 and 9) revealed the absence of the relevant 15q11-q13 deletion or duplication between BP2 and BP3. In CAL105 normal karyotype was found. In 4 subjects (Cases 5, 7, 8, and 10), frozen tissue and genetic data were not available.

Clinical Characteristics

Of the 9 subjects with dup(15), 7 (78%) were diagnosed with autism (Table 3). In 6 cases, autism was diagnosed clinically and was confirmed with postmortem ADI-R. In a 9-year-old boy (Case 1), autism was diagnosed with ADOS-G (Table 3). This case was reported as Case 2 in Mann et al (46). An 11-year-old boy (Case 3) revealed impairments consistent with the diagnosis of pervasive developmental disorder – not otherwise specified.

In the idiopathic autism group, all 10 subjects were diagnosed clinically as having an ASD. Postmortem ADI-R confirmed a classification of autistic disorder in 6 cases. Two subjects, an 8-year-old boy (Case 4) and a 52-year-old man (Case 10), were diagnosed with atypical autism or high-functioning autism. In a 5-year-old girl (Case 2), autism was diagnosed with ADOS-G. A 32-year-old man (Case 8) and a 52-year-old man (Case 10) were clinically diagnosed as having autism, but the ADI-R could not be conducted postmortem owing to the unavailability of a caregiver who could report on their behavior as a child.

Among the 9 examined subjects with dup(15) chromosomes, 7 individuals were diagnosed with seizures (78%) (Table 4). In 6 cases, death was sudden and unexplained in patients with epilepsy (SUDEP, 6/9 [67%]). In the 10 subjects with autism, epilepsy was diagnosed in 3 (30%) and death was seizure related in 1 (10%; Table 1). In a previously described cohort of 13 subjects with autism who were subject to postmortem examination (49), seizures were reported in 6 (46%) and death was seizure related in 4 (31%).

Brain Weight

The mean brain weight in dup(15) autism was 1,177 g, 300 g less than in idiopathic autism and 189 g less than in the control group (Table 1). Age-adjusted means for these 3 groups were 1,171, 1,474, and 1,378 g, respectively ($F_2 = 9.79$, p < 0.001). Post hoc tests showed the difference between the idiopathic and dup(15) groups with autism to be significant (Scheffé-corrected p = 0.001). The difference between the dup(15) and control groups was not significant, although suggestive (p = 0.06). The difference between the idiopathic and control groups was not significant.

Developmental Abnormalities in Autism Associated With dup(15) and Idiopathic Autism

Three major types of developmental changes, including (i) heterotopias, (ii) dysplastic changes, and (iii) defects of proliferation resulting in subependymal nodular dysplasia, were detected in both the dup(15) (Table, Supplemental Digital Content 1, http://links.lww.com/NEN/A323) and idiopathic autism (Table, Supplemental Digital Content 2, http://links.lww.com/NEN/A324) cohorts. In the control group,

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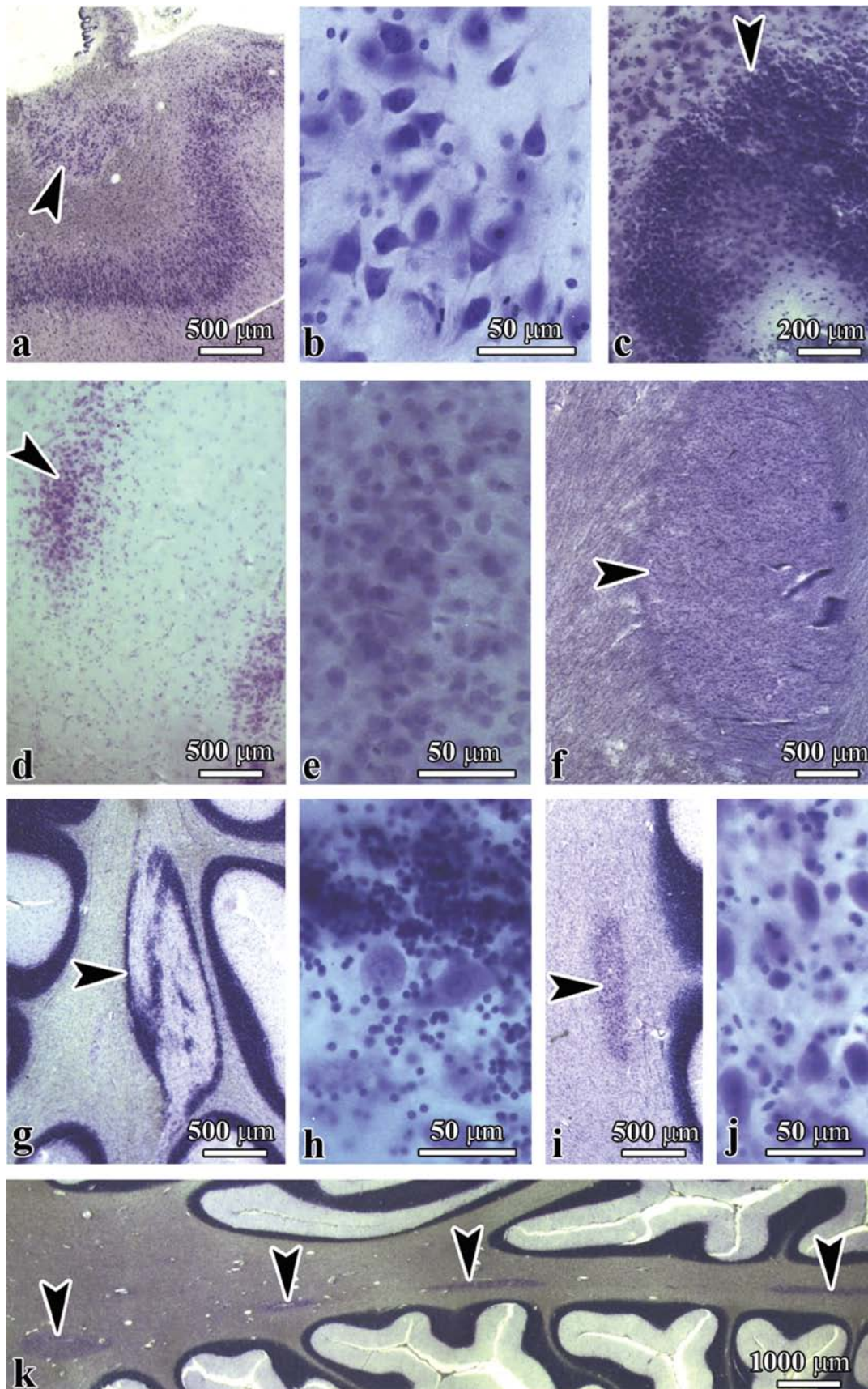
1 subject had small cerebellar subependymal heterotopias and 2 cases had dysplastic changes in the cerebellar flocculus and nodulus (Table, Supplemental Digital Content 3, http://links.lww.com/NEN/A325). Although all 9 dup(15) subjects and all 10 subjects with autism had developmental abnormalities, there were significant differences between the dup(15) autism and idiopathic autism cohorts in the number of developmental defects and their distribution (Table 5).

Heterotopias

Migration developmental abnormalities were detected as heterotopias in the hippocampal alveus, the CA4 sector, the dentate gyrus (DG) molecular layer, and the cerebral and cerebellar white matter (Fig. 1). A description of each is given below.

Heterotopias in the Hippocampus

A relatively large proportion of heterotopic cells in the alveus had the morphology of pyramidal neurons, although they were much smaller than neurons in the cornu Ammonis and were spatially disoriented. Heterotopias composed of neurons with the morphology of granule neurons of the DG were detected in the CA4 sector and in the molecular layer of the DG. Heterotopias in the alveus, CA4, and DG were found in 8 dup(15) subjects (89%), 1 subject with idiopathic autism (10%), and no control subjects. This difference between dup(15) autism and idiopathic autism cohorts ($p < 0.001$) and dup(15) and control subjects was highly significant ($p < 0.001$; Table 5). The difference between the idiopathic autism and control groups was not significant.

Heterotopias in the Cerebellar White Matter

The morphology of the cerebellar heterotopias reflected 2 types of migration defects. The presence of a mixture of granule and Purkinje cells suggests that clusters of cerebellar cortical neurons do not reach their destination site (Type 1). The second type of cerebellar heterotopia (Type 2) is composed of 1 type of cells with the morphology of cerebellar deep nuclei neurons. Both types of heterotopias were composed of neuronal nuclear marker–positive cells mixed with glial fibrillary acidic protein–positive astrocytes (not shown). In some cases, multiple Type 1 and 2 heterotopias were detected in the cerebellar white matter. In contrast to the significant difference in the prevalence of hippocampal heterotopias, there was no significant difference in the prevalence of heterotopias in the cerebellar white matter between the dup(15) (56%) and idiopathic autism (60%) groups (Table 5). However, the differences between the dup(15) autism and control groups ($p < 0.04$) and between the idiopathic autism and control groups ($p < 0.04$) were significant. Heterotopias in cerebral white matter were rare in both the dup(15) (1/9; 11%) and idiopathic autism (1/10; 10%) groups (Table, Supplemental Digital Content 1, http://links.lww.com/NEN/A323, and Table, Supplemental Digital Content 2, http://links.lww.com/NEN/A324).

Dysplasias

Dysplastic changes reflect focal microdysgenesis in the hippocampal DG and cornu Ammonis, the amygdala, and the cerebral and cerebellar cortices. Several types of dysplasia were detected in the DG, including hyperconvolution of the DG, duplication of the granular layer distorting the architecture of the molecular layer of the DG, irregular protrusions of the granular layer into the molecular layer, focal thinning and/or thickening of the granular layer, and fragmentation of the granular layer with the formation of isolated nests of granule cells (Fig. 2). The susceptibility of the DG to developmental abnormalities was several times more apparent in dup(15) syndrome than in idiopathic autism cases. They were detected in 8 (89%) of 9 subjects in the dup(15) group and in only 1 subject with autism (1/10, 10%; $p < 0.001$; Table 5). The number of different types of developmental abnormalities in the dup(15) group ranged from 2 per case in 4 subjects, to 3 per case in 3 subjects, and 5 types in 1 case. The total number of different types of dysplasia was 22 times greater in the dup(15) cohort than in the idiopathic autism cohort ($p < 0.001$). The difference between idiopathic autism (1 positive case) and the control group (no dysplastic changes in the DG) was not significant.

The spectrum of dysplastic changes in the cornu Ammonis comprised abnormal convolution of the CA1 sector, focal deficits of pyramidal neurons, and distortion of the shape, size, and spatial orientation of pyramidal neurons, clustering of dysplastic neurons in the CA1 sector, and many foci of severe microdysgenesis in the CA4 sector, with clustering of immature neurons (Figs. 3A–E). Dysplastic changes in the amygdala resulted in multiple irregular nests of 20 to 40 cells composed of relatively few small immature neurons and numerous oval or bipolar hyperchromatic neurons that were larger than normal amygdala neurons (Fig. 3F). Dysplastic changes in the cornu Ammonis were detected in 2 subjects with dup(15) syndrome and in 2 brains of subjects with autism (Table, Supplemental Digital Content 1, http://links.lww.com/NEN/A323, and Table, Supplemental Digital Content 2, http://links.lww.com/NEN/A324).

The presence of dysplastic changes in the cerebral cortex of 5 of the 10 subjects with idiopathic autism (50%) was in striking contrast to the absence of these changes in the dup(15) ($p < 0.03$) and control subjects ($p < 0.04$; Table 5). Three types of cerebral cortex dysplasia were found in the

FIGURE 1. Topography and morphology of heterotopias in the brains of individuals with duplications of chromosome 15q11.2-q13 (subjects with dup[15] and autism). **(A, B)** Heterotopia (**A,** arrowhead) in the alveus composed of bipolar, multipolar, and pyramidal-like neurons shown at higher magnification in **B**. **(C–E)** Heterotopias composed of cells with the morphology of granule neurons in the CA4 sector (**C,** arrowhead) and in the molecular layer (arrowhead) of the dentate gyrus **(D)**; higher magnification is shown in **E**. **(F)** Heterotopia (arrowhead) in the cerebral white matter. **(G–J)** Heterotopias in the cerebellum consist of mixed components of the cerebellar cortex (**G,** arrowhead), including granule and Purkinje cells **(H)**; one is composed of one type of neuron (**I,** arrowhead), with the morphology of cells of cerebellar deep nuclei **(J)**. **(K)** Multifocal heterotopias of both types in cerebellar white matter (arrowheads). **(A–C, F)** dup(15), Case 2; **(D, E)** dup(15), Case 6; **(G–J)** dup(15), Case 5; **(K)** idiopathic autism, Case 5.

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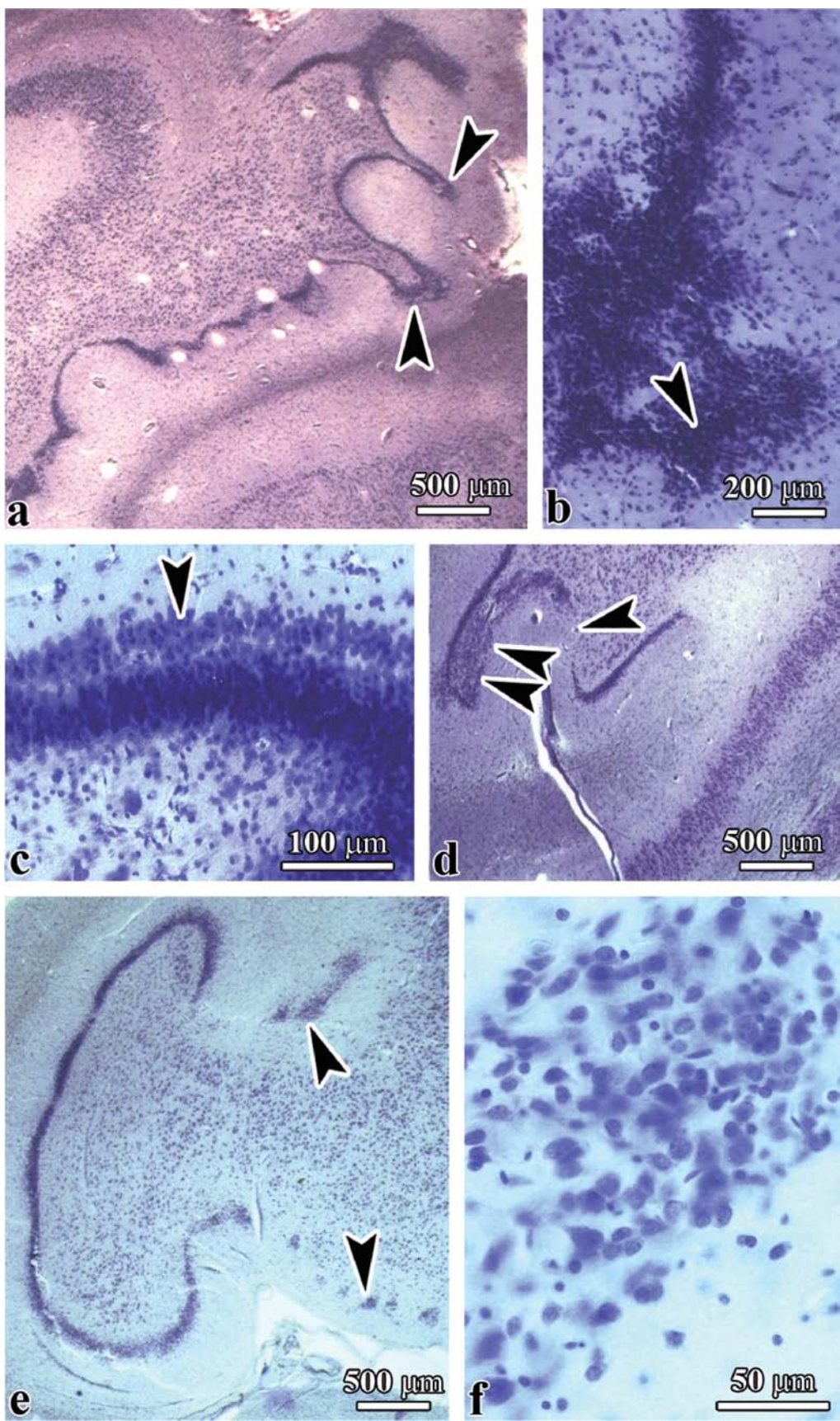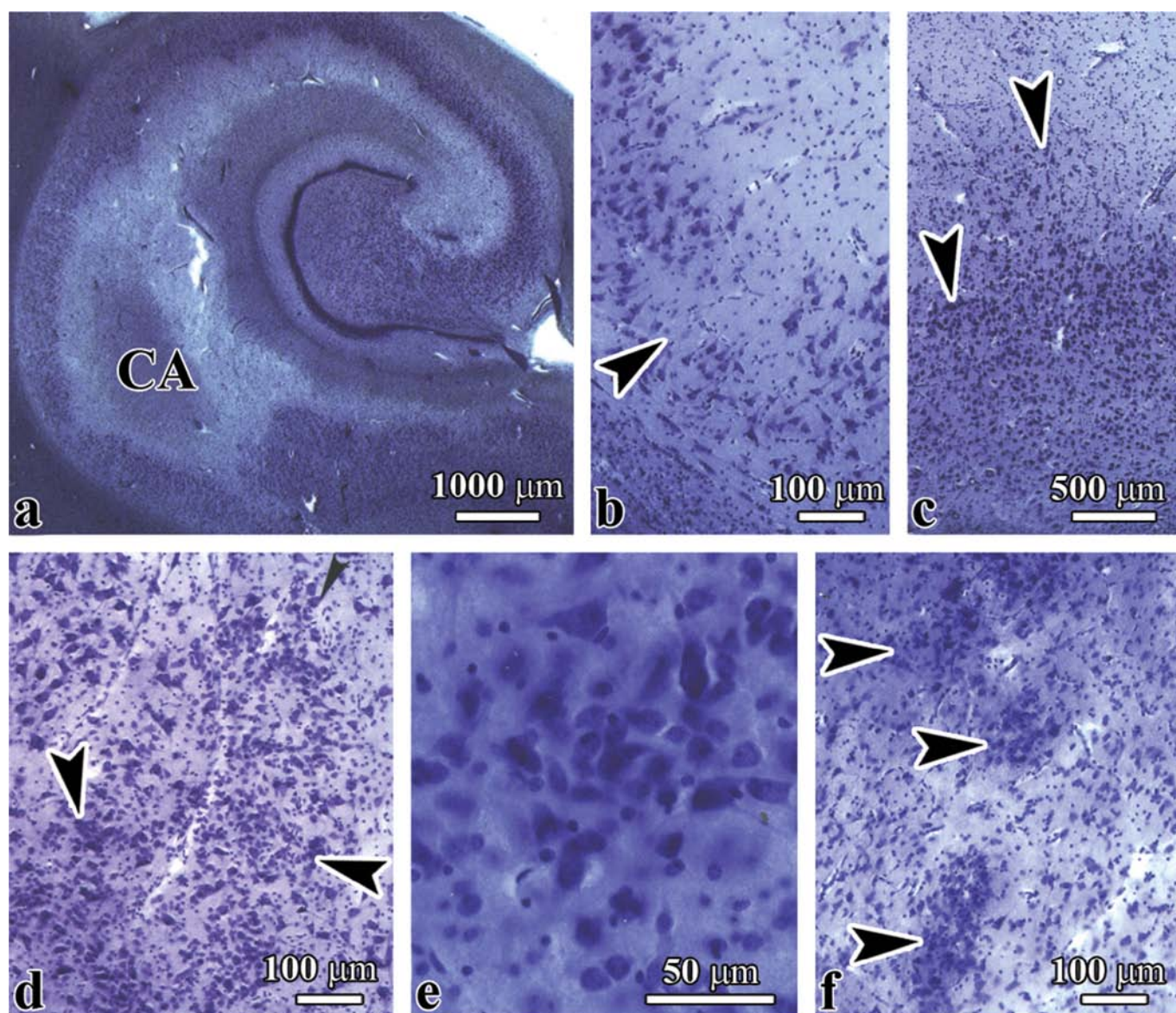
FIGURE 3. Multiple dysplastic changes in the cornu Ammonis (CA) and amygdala in an 11-year-old boy with hexasomy of chromosome 15q11.2q13 (Case 3). **(A–C)** There is abnormal convolution of the CA1 sector **(A)** with focal microdysgenesis of the pyramidal layer **(B, arrowhead)** and clustering of disoriented polymorphic neurons **(C, arrowheads)**. **(D, E)** Marked multifocal microdysgenesis in the CA4 sector **(D, arrowheads)**, with clustering of a mixture of small and large polymorphic neurons **(E)**. **(F)** Multifocal microdysgenesis (arrowheads) in the amygdala is composed of small immature neurons and neurons that are larger than normal amygdala neurons.

idiopathic autism group: focal polymicrogyria, multifocal cortical dysplasia, and bottom-of-a-sulcus dysplasia (Supplemental Digital Content 2, http://links.lww.com/NEN/A324).

Focal polymicrogyria, which reflects a gyrification defect, was found in the frontal lobe in an 8-year-old boy with

autism diagnosed with ASD (Case 4), which resulted in local abnormal folding of the cortex with formation of numerous small and irregular microgyri and distortion of the cortical thickness and vertical/horizontal cytoarchitecture (Fig. 4A). The most common developmental abnormality was cortical

FIGURE 2. Six types of dysplastic changes in the dentate gyrus of subjects diagnosed with duplications of chromosome 15q11.2-q13 (dup[15]) syndrome. **(A–F)** Hyperconvolution of the dentate gyrus within the hippocampal body **(A)**, irregular large protrusions of the granular layer **(B)**, duplication of the granular layer **(C)**, focal thinning and discontinuity of granular layer (arrowhead), and thickening of the granular layer confirmed by examination of serial sections **(D, 2 arrowheads)**, hippocampal malrotation and granular layer fragmentation into small clusters of cells of irregular shape and variable size **(E, F)**. **(A)** dup(15), Case 8; **(B)** dup(15), Case 7; **(C, E, F)** dup(15), Case 3.

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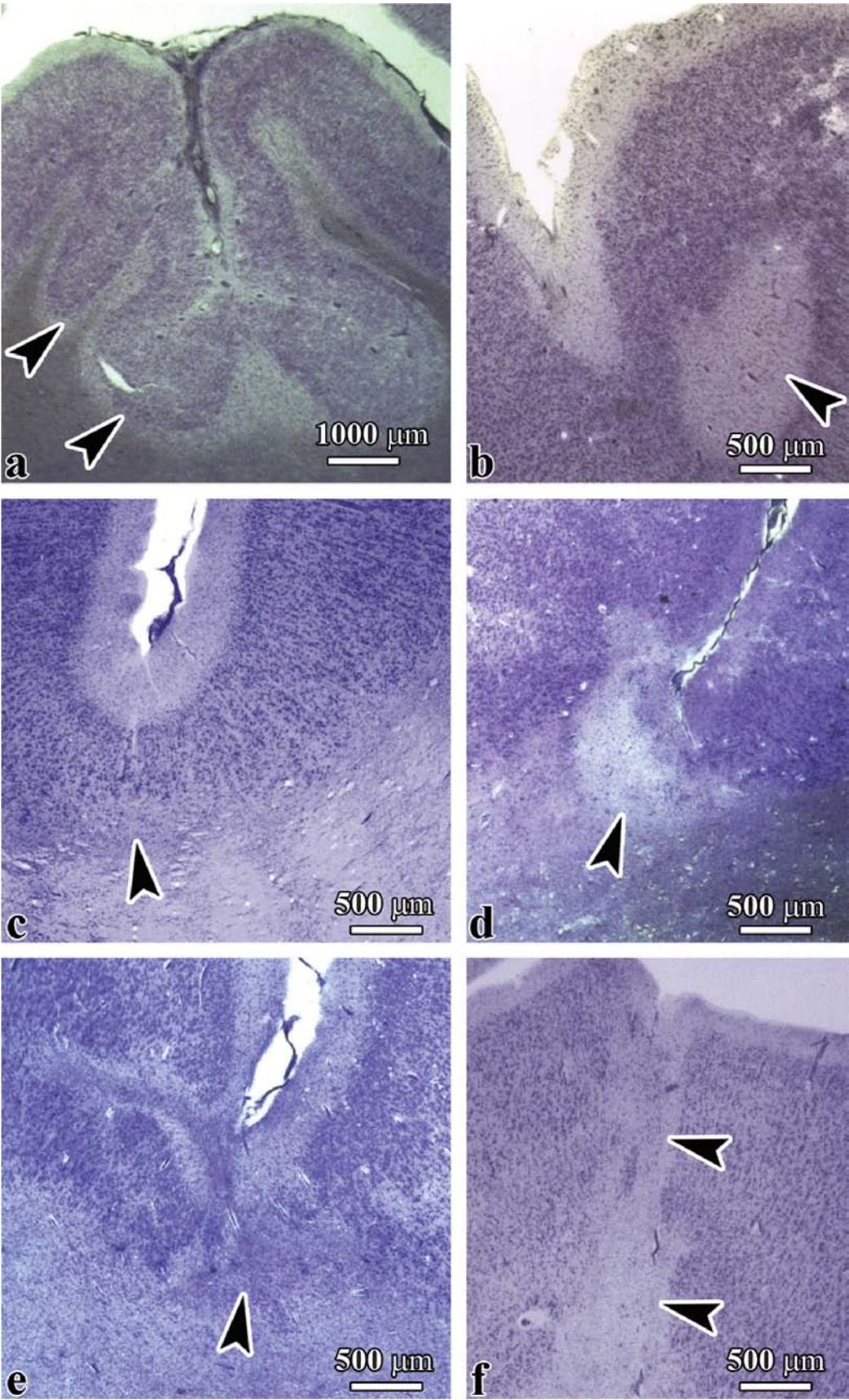
dysplasia with focal hypocellularity or acellularity and loss of cortical vertical and horizontal cytoarchitecture (Fig. 4B).

Another observed gyrification defect was multifocal bottom-of a-sulcus dysplasia with selective changes in the deepest layer, expansion of dysplastic changes to 2 to 3 deep layers of the cortex, or affecting the entire thickness of the cortex. This developmental abnormality was most often seen in the superior frontal and temporal gyrus, the Heschl gyrus, the middle temporal gyrus, the insula, and the parahippocampal gyrus in a 5-year-old girl diagnosed with idiopathic autism (Case 2; Figs. 4C–F).

Three types of dysplastic changes were found in the cerebellum of the dup(15) subjects and the subjects with autism. These included dysplasia in parts of the nodulus and flocculus, vermis dysplasia, and focal polymicrogyria (Table, Supplemental Digital Content 1, http://links.lww.com/NEN/A323, and Table, Supplemental Digital Content 2, http://links.lww.com/NEN/A324). In the nodulus and flocculus, dysplasia resulted in total spatial disorganization of the granular, molecular, and Purkinje cell layer; only a few small abnormally branched Purkinje cells were found to be dispersed among the granule cells in the affected areas. There were many interindividual differences observed in the nodulus or flocculus volume affected by dysplastic changes. Cerebellar dysplasia was commonly observed in both cohorts. Nodulus dysplasia was present in 7 (87%) of 8 dup(15) subjects and in 6 (75%) of 8 subjects with autism (Table, Supplemental Digital Content 1, http://links.lww.com/NEN/A323, and Table, Supplemental Digital Content 2, http://links.lww.com/NEN/A324). Flocculus dysplasia was detected in 6 (75%) of 8 dup(15) subjects, in 4 (50%) of 8 subjects with autism, and in 1 (14%) of 7 control subjects (Table 5). The difference between the groups with autism was not significant, but the difference between the dup(15) autism and control group was significant (p < 0.05).

Subependymal Nodular Dysplasia

Nodular dysplasia was found in the brain of a 15-year-old adolescent girl diagnosed with dup(15) (Case 5). This consisted of a single large nodule in the wall of the temporal horn of the lateral ventricle and numerous nodules in the wall of the lateral ventricle in the occipital lobe. Subependymal nodular dysplasia was also detected in the brain of a 39-year-old woman diagnosed with dup(15) (Case 9; Table, Supplemental Digital Content 1, http://links.lww.com/NEN/A323). There were numerous subependymal nodules less than 1 to 3 mm in diameter in the wall of the occipital horn of the lateral ventricle in a 32-year-old subject with idiopathic autism (Case 8; Table, Supplemental Digital Content 2, http://links.lww.com/NEN/A324). These were composed of dysplastic neurons with a partially modified morphology of pyramidal, multipolar, or bipolar neurons and oval medium and small cells. In all 3 cases, the nodules were free of oval or polygonal giant cells or ballooned glial cells. Examination of the thalamus, caudate, putamen, nucleus accumbens, and globus pallidus did not reveal developmental qualitative abnormalities in these cohorts.

Differences Between the Global Pattern of Developmental Abnormalities in dup(15) and Autism

Although all dup(15) and subjects with autism had developmental abnormalities, the number of different types of developmental alterations detected in the brains of the dup(15) group was, on average, 2.3 times greater (6.9 per case; n = 9) than in the subjects with autism (3 per case; n = 10). Analysis of developmental alterations in 13 subjects with autism previously reported (49) revealed developmental abnormalities in the brains of all subjects with autism and a similar prevalence of alterations.

Other Neuropathologic Changes

Selective and marked neuronal loss without gliosis was found in the pyramidal layer in the CA1 sector in the brain of a 10-year-old boy with epilepsy (dup[15]; Case 2). Pathologic alterations extended from the head to the tail of the hippocampal formation, with loss of neurons in the range of 80% in the head and 50% in the body and tail. These findings might be the result of severe and frequent seizures.

An area of marked subpial gliosis was found within a sulcus between the inferior frontal and the orbital gyri in the brain of a dup(15) female with epilepsy and seizure-related asphyxia at the age of 26 years (Case 8). Almost complete focal loss of the granular layer was associated with gliosis, thickening of the affected molecular layer, degeneration of astrocytes, and deposition of many corpora amylacea. These findings most likely represent Chaslin gliosis, indicative of epilepsy-related brain damage. This pathologic condition coexisted with hyperconvolution of the DG, focal thinning, and duplication of the granular cell layer, considered developmental abnormalities contributing to abnormal electrical activity and seizures.

DISCUSSION

Knowledge of the clinical phenotype and genetic factors in autism is based on examination of thousands of individuals with idiopathic autism; however, between 1980 and 2003, only 58 brains of individuals with idiopathic autism were examined postmortem (50). Knowledge of the clinical and genetic characteristics of the dup(15) syndrome is based on examination between 1994 and 2006 of approximately 160 cases (47, 51–54), but the neuropathology of dup(15) with and without autism has not been studied. Results of the application of an extended neuropathologic protocol were previously reported for 13 brains of subjects with idiopathic autism (49). The current study characterizes qualitative neuropathologic changes in the brains of 9 individuals with

FIGURE 4. Cerebral cortex dysplasia autism spectrum disorder (ASD). **(A, B)** Polymicrogyria (**A**, arrowheads), focal hypocellularity or acellularity with lack of vertical and horizontal cortical organization (**B**, arrowhead) in an 8-year-old boy with ASD. **(C–F)** Bottom-of-a-sulcus dysplasia in a 5-year-old girl with idiopathic autism (Case 2). Focal dysplasia was limited to deep cortical layers **(C)**, affected all layers except the molecular layer **(D)**, and caused cortex fragmentation **(E)** or disruption of cortical ribbon continuity (**F**, arrowheads).

dup(15), including 7 diagnosed as having an ASD (78%). This autism prevalence is in the highest range reported in clinical studies. The association with autism in some of the earlier individual reports (i.e. 4 [33%] of 12 [55] or 6 [36%] of 17) was not based on use of standardized screening (56). A standardized assessment of autistic manifestations in 29 children and adults with a supernumerary idic(15) detected in 20 individuals (69%) with a high probability of ASD (29). All studies reported a significant variability in the autistic phenotype, severity of autistic features, delayed development and/or ID, and seizures among subjects with dup(15) (15).

Major Neuropathologic Differences Between dup(15) Autism and Idiopathic Autism

Numerous studies indicate that autism is associated with a short period of increased brain size (57–59) and more neurons (60). Macrocephaly was detected in 37% of children with autism younger than 4 years (61) and in 42% of the 19 twins diagnosed with idiopathic autism younger than 16 years (6). Postmortem studies (62, 63) and imaging studies (64) also provided converging evidence of increased brain volume in autism. Microcephaly has been observed in only 15.1% of 126 children with autism aged 2 to 16 years (65) and is usually associated with severe pathology (66, 67), ID, and other medical disorders (65).

This study revealed a high prevalence of microcephaly in the dup(15) autism cohort examined postmortem, that is, the mean weight of the brains of subjects with dup(15) autism was 300 g less than that of subjects with idiopathic autism and 189 g less than that in the controls ($p < 0.001$ for both). The characteristics of head circumference and brain volume in dup(15) cohorts have been studied less comprehensively than in idiopathic autism, but published reports also show a strong prevalence of microcephaly. A summary of records from 107 supernumerary inv dup(15) cases revealed that only 3 subjects had macrocephaly (2.8%), but 6 times more cases (n = 18, 16.8%) had microcephaly (15). Battaglia (68) detected microcephaly in radiologic evaluations of 1 in 4 subjects with dup(15) whose age ranged from 4 to 8 years. These data suggest the failure of mechanisms controlling brain growth in autism, resulting in the prevalence of macrocephaly in idiopathic autism and of microcephaly in dup(15) autism.

Our extended neuropathologic protocol revealed several striking differences between the pattern of developmental alterations in dup(15) autism and idiopathic autism. Neuronal migration defects in the hippocampus resulting in heterotopias in the alveus, CA4, and DG were 8 times more common in dup(15) autism (89% of subjects) than in idiopathic autism (10%; $p < 0.001$). The second developmental abnormality distinguishing dup(15) autism from idiopathic autism was the dysplasia that occurred 8 times more often in the DG of subjects with dup(15) and the different types of developmental abnormalities that occurred 22 times more often in the DG of subjects with dup(15) ($p < 0.001$). The third factor differentiating these 2 cohorts was the absence of cerebral cortex dysplasia in dup(15) autism and the presence of this pathology in 50% of subjects with idiopathic autism

($p < 0.03$). The increased number of developmental alterations and the topographic differences suggest significant differences between mechanisms contributing to abnormal neuronal migration and altered cytoarchitecture in these 2 cohorts.

Linkage and gene mapping analysis, molecular reports, and clinical studies revealed the link between de novo, maternally derived proximal 15q chromosome alterations, and autism (13, 15, 27, 33, 56, 69–72). This postmortem study suggests that neuropathologic profile with microcephaly and multiple focal developmental defects is another marker of maternally derived proximal 15q chromosome alterations contributing to autistic phenotype.

Although ASD and epilepsy are heterogeneous disorders, they often occur together. This may indicate that these disorders share some underlying mechanisms and that epileptogenesis affects brain structure and function, which modify the clinical manifestations of autism. Approximately 30% of children with autism are diagnosed with epilepsy and 30% of children with epilepsy are diagnosed with autism (73). Significant cognitive impairment is present in approximately 50% of all individuals who have autism (74). Early onset of seizures contributes to clinical regression, enhanced severity of autistic phenotype, and enhanced mortality. The rate of death among subjects with autism is 5.6 times higher than expected (75), and epilepsy- and cognitive impairment–related accidents account for most of the deaths (75–78). The diagnosis of epilepsy in 78% (7/9) of postmortem-examined individuals with dup(15) indicates that epilepsy is an important component of the clinical phenotype in most individuals diagnosed with dup(15).

Microcephaly might be one of several indications of brain immaturity that increases the risk of epilepsy. The immature brain exhibits increased excitation, diminished inhibition, and increased propensity for seizures in infancy and early childhood (79). The reduced volume of neurons in most subcortical structures and some cortical regions in the brains of 4- to 8-year-old children with autism seems to reflect brain immaturity in early childhood (80). In the normally developing brain, maturation of the frontal and temporal cortex is associated with differential expression of 174 genes; however, none of these genes are differentially expressed in ASD (81). Altered development of neurons resulting in brain immaturity may contribute to an increased tendency for the seizures and epileptogenesis observed in the examined cohort. Very early onset of intractable epilepsy (at 10, 8, and 10 months, respectively) was reported in all 3 of the youngest individuals diagnosed with dup(15), who died as a result of SUDEP at 9, 10, and 11 years. These findings suggest that very early onset of seizures and very severe seizures may increase the risk of SUDEP in this cohort. Severe ID in all 3 of these individuals indicates that brain immaturity and a profound ID are another SUDEP risk factor in the examined cohort of subjects with dup(15). The combination of all these factors may contribute to death at a very early age ($\sim$10 years).

Sudden unexpected death in childhood (SUDC) is the sudden death of a child older than 1 year that, despite a review of the clinical history, circumstances of death, and complete autopsy with appropriate ancillary testing, remains unexplained.

It occurs throughout childhood (<18 years) but occurs most commonly between the ages of 1 and 4 years (82, 83). Sudden unexpected death in childhood is an unexpected and unexplained death that occurs in patients with known epilepsy, including children, and is typically associated with sleep (84, 85). Kinney et al (86) reported several types of hippocampal anomalies in SUDC cases, including hyperconvolution of the DG, focal duplication of the DG, granular nodular heterotopia, abnormal folding of the subiculum, and focal clustering of pyramidal neurons in the cornu Ammonis. These developmental anomalies are considered a cause of seizure-related autonomic and/or respiratory dysfunction and sudden death (87–89). Kinney et al (86) proposed that these anomalies represent an epileptogenetic focus that, when triggered by fever, trivial infection or minor head trauma at a susceptible age, may result in unwitnessed seizure, cardiopulmonary arrest, and sudden death. The presence of these developmental anomalies in the examined brains of individuals with dup(15) may explain SUDEP in 5 cases and SUDC in 2 other cases. The presence of these changes in the hippocampus of subjects with dup(15) who died of causes other than SUDEP or SUDC suggests that they also were at higher risk for sudden unexpected death. Collectively, these data indicate that the risk of SUDC is much higher in the dup(15) cohort than in the general population with SUDC in which there is an overall rate of 57 of 100,000 deaths per year (90).

Microdysgenesis is not specific for dup(15), epilepsy, or autism; it has been reported in ID (91), psychosis (92), and dyslexia (93), as well as in some control subjects. None of these developmental alterations can be considered pathognomonic of an ''epileptic'' brain (83), but changes in the cohort affected by dup(15) or idiopathic autism reveal significant differences. This indicates that not a single lesion but, instead, a complex pattern of developmental defects distinguishes these subjects. A 2.3-fold higher prevalence of these developmental abnormalities, 2.3 times higher prevalence of epilepsy, and 6 times higher prevalence of epilepsy-related death in the dup(15) cohort compared with the idiopathic autism group suggest that the mechanisms leading to developmental structural alteration in the hippocampal formation are a major contributor to epilepsy and SUDEP/SUDC in dup(15). These seem to be a much weaker contributor to epilepsy and SUDEP in idiopathic autism. The results presented in this report reinforce the hypothesis that additional copies of the critical 15q11-q13 region are causally related to the autism phenotype and developmental abnormalities contributing to epilepsy and to an increased risk of SUDEP and SUDC. Future studies of the expression and distribution of proteins encoded by $GABA_A$ receptor subunit genes ($\alpha5$, $\beta3$, and $\gamma3$) and the gene for juvenile epilepsy located near D14S165 on chromosome 15 may explain the role of duplication or triplication of these genes in autism and the enhanced susceptibility to seizures in dup(15) syndrome.

In conclusion, despite the common clinical diagnosis of autism in the dup(15) and idiopathic cohorts, significant differences in brain growth and focal developmental defects of neuronal migration and cytoarchitecture indicate that the dup(15) autistic phenotype is a product of unique genetic, molecular, and neuropathologic alterations.

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Supplemental Table 1. **Developmental Alterations in the Brain of Subjects Diagnosed with dup (15)**

Case #	Sex, Age	Developmental Alterations. [Other neuropathological changes listed in square brackets].
1	M, 9	Heterotopias in the alveus and stratum oriens in the hippocampal head and body (1). Heterotopia in the cerebellar white matter (2). Dysplasia of the dentate gyrus with local granule cell layer thinning (3) and fragmentation into numerous separate neuronal islands (4). Dysplasia in the cerebellar nodulus (5).
2	M, 10	Heterotopias in the alveus (1). Dentate gyrus granule cell layer discontinuity (2) and hyperconvolution (3). Heterotopias in the cerebral white matter (4). Dysplasia in the cerebellar nodulus (5) and flocculus (6). [Severe neuronal loss in the CA1].
3	M, 11	Multiple foci of microdysgenesis in amygdala (1). Heterotopia in the hippocampal alveus (2). Fragmentation of the granule cell layer of the dentate gyrus (3) and focal duplication of the granule cell layer (4). Abnormal convolution and malrotation of the cornu Ammonis (5). Focal microdysgenesis of the pyramidal layer with a deficit of pyramidal neurons (6) and clustering of disoriented immature neurons in CA1 (7). Severe multifocal microdysgenesis in the CA4 sector with clustering of small and large polymorphic neurons (8). Two heterotopias built of granule and Purkinje cells in the cerebellar white matter (9). Dysplasia in the cerebellar nodulus (10) and flocculus (11).
4	F, 15	Heterotopia in the alveus (1). Dysplasia in cerebellar nodulus (2) and flocculus (3).
5	F, 15	Heterotopia in the alveus (1). Dentate gyrus microdysgenesis with focal duplication or triplication of granule cell layer (2), discontinuity of granule layer (3), and granule cell layer several-fold thickening (4). Large subependymal nodular dysplasia in lateral ventricle (5). Multifocal subependymal nodular dysplasia within the wall of the ventricle in the occipital lobe (6). Cerebellar developmental alterations with focal polymicrogyria (7), small heterotopia built of cells resembling neurons of the cerebellar deep nuclei (8), and large heterotopia build of granule neurons and Purkinje cells (9).
6	M, 20	Heterotopia in the dentate gyrus molecular layer (1), focal granule layer thinning and fragmentation (2), and duplication (3). Dysplasia in the cerebellar nodulus (4) and flocculus (5). [Severe hypoxic encephalopathy].
7	M, 24	Dentate gyrus hyperconvolution (1). Dentate gyrus granule cell layer duplication (2), irregular large protrusions (3), focal thinning and discontinuity (4), and focal fragmentation (5). Two large heterotopias in the CA4 sector built of cells with morphology of granule layer neurons (6). Multi-focal microdysgenesis in the CA4 sector with large and small clusters of immature small neurons (7). Two types of heterotopias in the cerebellar white matter built of neurons resembling cerebellar nuclei (8) or altered cerebellar cortex (9). Dysplasia in the cerebellar nodulus (10) and flocculus (11).
8	F, 26	Hyperconvolution of the dentate gyrus (1). Focal thinning (2) and duplication (3) of the dentate gyrus granule cell layer. Cerebellar developmental changes with multiple focal microdysgenesis in the vermis (4), dysplasia in the cerebellar nodulus (5) and flocculus (6), and subcortical and periventricular heterotopias in the cerebellar white matter (7). [Seizure-related focal loss of neurons in the second layer of the orbital and frontal inferior gyrus with Chaslin gliosis].
9	F, 39	Hyperconvolution of the dentate gyrus (1). Focal thinning (2) and thickening (3) of the granule cell layer in the dentate gyrus. Heterotopia in the CA4 sector (4). Heterotopias in the CA1 sector (5). Subependymal nodular dysplasia (6). [Multifocal calcification in basal ganglia and white matter].

Supplemental Table 2. Developmental Alterations in the Brains of Subjects Diagnosed with Idiopathic Autism

Case #	Sex, Age	Developmental Alterations. [Other neuropathological changes listed in brackets]
1	M, 2	Frontal lobe dysplasia type I with abnormal thickening of the cortex, focal loss of the molecular and thickening of the granule layer, and loss of the vertical and horizontal organization (1). Duplication of the dentate gyrus granule cell layer (2). Multifocal cerebellar heterotopias (3). Dysplasia in the cerebellar nodulus (4) and flocculus (5). [Severe multifocal necrosis caused by hypoxic-ischemic changes associated with mechanism of death].
2	F, 5	Distorted cortical gyrification with severe multifocal neocortical dysplasia in the superior frontal and temporal gyrus, insula, Heschl gyrus, middle temporal g, and parahippocampal gyrus. Microdysgenesis of deep cortical layers especially often and severe at the bottom of cortical sulcus (2). Cerebellar floccular dysplasia (3).
3	M, 5	Multifocal cortical dysplasia type I in the parietal and occipital lobes with focal hypo- or acellularity and loss of cortical cytoarchitecture (1). Cerebellar floccular (2) and nodular (3) dysplasia.
4	M, 8	Frontal lobe focal polymicrogyria (1). Dysplasia with abnormal lamination in frontal and temporal cortex (2). Heterotopia in alveus (3). Heterotopia in the subependymal area in cerebellar white matter (4). Cerebellar floccular (5) and nodular (6) dysplasia.
5	M, 9	Multiple heterotopias within the cerebellar white matter (1). Cerebellar nodular dysplasia (2).
6	M, 11	Focal ectopic large neurons within folial subcortical white matter (1). [Cardiac arrest encephalopathy with generalized ischemic brain damage].
7	M, 29	Developmental focal neuronal deficit in the temporal cortex (hypocellularity) (1). Two heterotopias in the cerebellar white matter (2). Cerebellar nodular dysplasia (3). [Mild vasculitis].
8	M, 32	Very prominent subependymal nodular dysplasia in the wall of the occipital horn of lateral ventricle (no giant or bizarre cells) (1). Heterotopia in the periventricular white matter near frontal horn of the lateral ventricle (2). Hyperconvolutions of the CA1 and dentate gyrus (3). Heterotopia in the cerebellum (4). [Glioblastoma multiforme of the right temporo-occipital-parietal area].
9	M, 51	Nodular dysplasia (1). [Cardiac arrest encephalopathy with generalized ischemic brain damage].
10	M, 52	Hyperconvolution and distortion of laminar organization of the CA1 (1). Distortion of laminar organization of the subiculum proper (2).

Supplemental Table 3. Neuropathology in the Control Group

Case #	Sex	Age, Years	Developmental Alterations [Other Neuropathological Changes Listed in Brackets]
1	F	8	[Focal loss or deficit of neurons without glial response in the caudate nucleus and temporal cortex]
2	M	14	No pathological changes
3	M	14	No pathological changes
4	M	32	Subependymal heterotopia in the cerebellum (1). [Perivascular calcifications in the hippocampus and in the globus pallidus]
5	F	33	No pathological changes
6	F	43	Cerebellar nodular dysplasia (1).
7	M	47	Cerebellar nodular (1) and floccular (2) dysplasia.

Clinicopathological Stratification of Idiopathic Autism and Autism Associated with Duplications 15q11.2-q13

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Abstract

Postmortem studies of brains of individuals with idiopathic autism and duplications 15q11.2-q13 autism identify a cluster of neuropathological features differentiating these cohorts. They show a need for both sub-classification of autism according to etiology, clinical presentation, and neuropathology, and a commonality of clinical and neuropathological traits justifying autism diagnosis. The features differentiating these cohorts include: (a) maternal origin dup(15), (b) autism in 78% of subjects, (c) more severe clinical phenotypes, with intellectual deficit (100%), early-onset of severe or intractable seizures in 78% of subjects, and increased to 67% prevalence of sudden unexplained death, (d) high prevalence of microcephaly, with mean brain weight 300g less than in idiopathic autism, (e) several-fold increase in the number of developmental abnormalities, including defects of migration and dysplastic changes, especially numerous in the hippocampal formation, and (f) significant increase of the intraneuronal amyloid load, reflecting enhanced amyloid-β precursor protein processing with α-secretase.

Genetic factors in autism. In 1977 Folstein and Rutter (Folstein and Rutter, 1977) demonstrated a striking difference in concordance rates of autism between monozygous and dizygous twins. The studies that followed revealed close to 90% monozygous concordance rates for autism spectrum disorder (ASD) and very low concordance rates for dizygotic twins (Bailey *et al.*, 1995), showing a significant role of genetic factors in autism etiology (Ritvo *et al.,* 1985; Smalley *et al.*, 1988, Steffenburg *et al.*, 1989; Folstein and Piven, 1991; Rutter *et al.*, 1990a,b; Lotspeich and Ciaranello, 1993). Recent studies demonstrate contribution of both genetic and environmental factors to autism and ASD. Liu *et al.* (2010) revealed 57.0% and 67.2% concordance rates for monozygotic males and females, respectively, 32.9% concordance rates for same sex dizygotic twins, and 9.7% recurrence risk for siblings, whereas Hallmayer *et al.* (2011) demonstrated moderate, 37% and 38% genetic heritability for autism and ASD, respectively, and 55% contribution of shared environmental factors to autism and 55% to ASD.

Genetic basis has been revealed for less than 15% of autism cases, whereas no single genetic cause explains more than 2% (Abrahams and Geshwind 2008, Wang *et al.*, 2009). Chromosomal abnormalities, especially large chromosomal anomalies, such as unbalanced translocations, inversions, rings, and interstitial deletions and duplications, were detected in 1.7% to 4.8% of subjects diagnosed with ASDs (Lauritsen *et al.*, 1999; Wassink *et al.*, 2004). They are identified as duplications of 15q [dup(15)], deletions of 18q, Xp, 2q, and such sex chromosome aneuploidies as 47,XYY and 45,X (Gillberg, 1998; Reddy, 2005). Autism is diagnosed in 69% of subjects with maternal origin duplications 15q11.2-q13 (dup15) (Rineer *et al.*, 1998), in 15% to 28% of individuals with fragile X syndrome (FXS) (Hagerman, 2002), and in 7% of people with Down syndrome (DS) (Kent *et al.*, 1999).

Duplications of chromosome 15q11q13. The imprinted chromosome region 15q11q13 is known for its instability, resulting in the DNA repeats or deletions associated with several syndromes. Prader Willi syndrome (PWS) is predominantly the result of a paternal deletion of the small nuclear ribonucleoprotein polypeptide N (SNRPN) gene in 15q11q13 (Ozcelik *et al.*, 1992), whereas the Angelman syndrome (AS) is most often a result of maternal deletion of the ubiquitin-protein ligase E3A (UBE3A) gene (Knoll *et al.*, 1993). Subsets of subjects with PWS or AS have been reported to exhibit autistic-like behavior (Arrieta *et al.*, 1994; Demb and Papola, 1995; Dykens and Kasari, 1997; Penner *et al.*, 1993; Steffenburg *et al.*, 1996; Summers *et al.*, 1995). Chromosomal abnormalities of the proximal 15q region belong to the most common genomic aberrations detected in autistic disorder

probands (Arrieta *et al*., 1994; Baker *et al*., 1994; Bundey *et al*., 1994; Cook EH *et al*., 1997; Flejter *et al*., 1996; Gillberg *et al*., 1991; Hotopf and Bolton, 1995; Kerbeshian *et al*., 1990; Martinsson *et al*., 1996; Schroer *et al*., 1998; Weidmer-Mikhail *et al*., 1998; Wolpert *et al*., 2000a,b). These abnormalities were found in up to 3% of subjects diagnosed with autism. An especially strong association with autism was revealed in duplications in the range of 8 to 12 Mb derived from the maternal chromosome (Cook EH *et al*., 1997; Dawson *et al*., 2002). Interstitial triplications [int trp(15)] are relatively rare but have invariably been associated with a severe phenotype, including intellectual deficit (ID), ASD, and seizures. A few subjects have been diagnosed with duplications of paternal origin; however, they were described as clinically unaffected (Bolton *et al*., 2001; Cook EH *et al*., 1997; Mohandas *et al*., 1999; Schroer *et al*., 1998) or affected but without ASD (Mao *et al*., 2000; Mohandas *et al*., 1999). In only one subject was paternal origin dup(15) associated with ASD (Bolton *et al*., 2004). Because only maternally inherited aberrations of chromosome 15q11q13 have been reported to be associated with severe clinical phenotype, one may assume that the copy number of maternal genes within this genomic region contributes to alterations of brain development and the autistic phenotype.

Gene expression in dup(15). Postmortem studies of the brain reveal that chromosome duplications 15q11-13 are associated with epigenetic alterations in gene expression that are not predicted from copy number (Hogart *et al*., 2008). Whole-genome expression profiling of lymphoblast cell lines derived from individuals with autism and isodicentric 15 [idic(15)] revealed 112 transcripts that are significantly dysregulated in samples from subjects with duplications. However, only four of a total of 80 genes located within the duplicated area of chromosome15 were found to be up-regulated, including 1.5- to 2.0-fold up-regulation of ubiquitin protein ligase E3A (*UBE3A*; 15q11-q13) and 1.89-fold increase of *HERC2*. Baron *et al.* (2006) concluded that the majority of changes are not due to increased gene dosage in a critical chromosome 15 region, but represent potential down-stream effects of this duplication, including two down-regulated genes: *APP* encoded by a gene on chromosome 21, and *SUMO1* encoded by a gene on chromosome 2. Several functional categories were identified as associated with macromolecular catabolic processes, including the ubiquitin-dependent protein catabolism. The increase of UBE3A protein level may indicate dysregulation of ubiquitin-mediated proteasome pathway in cells with dup(15), resulting in enhanced ubiquitination of proteins for non-lysosomal degradation/disposal in response to genotoxic insult. Down-regulation of SUMO1, a

ubiquitin-like molecule, may indicate other forms of dysregulation of cell catabolic processes leading to decreased cell sensitivity to apoptotic stimuli and tolerance to DNA damage (Baron *et al*., 2006).

The γ-aminobutyric acid type$_A$ (*GABA$_A$*) receptor subunit genes (*α5, β3,* and *γ3*) are located in the susceptibility segment of duplicated chromosome 15 (Bass *et al*., 2000; Buxbaum *et al*., 2002; Cook *et al*., 1998; Menold *et al*., 2001). GABA is the main inhibitory neurotransmitter binding to a complex of GABA$_A$ receptors. Polymorphisms in the *GABA$_A$-β3* receptor subunit are associated with autism (Cook *et al*., 1998; Martin *et al*., 2000). Moreover, differential methylations in the *GABA$_A$* gene (Bittel *et al*., 2005; Gabriel *et al*., 1998; Hogart *et al*., 2007; Meguro *et al*., 1997;) may result in epigenetic modifications and modifications of clinical phenotypes in dup(15)/autism.

Clinical characteristics. Between 1994 and 2008, approximately 160 patients diagnosed with inverted (inv) dup(15) were characterized (Battaglia, 2008; Dennis *et al*., 2006; Wang *et al*., 2004; Webb, 1994; Webb *et al*., 1998; Wolpert *et al*., 2000a,b;). A similar male:female ratio (3:1) has been reported in idiopathic autism (Bryson, 1996) and in probands with dup (15q) (Wolpert *et al*., 2000a).

Clinicopathological correlations in dup(15) cohorts with considerable variations in the breakpoints, copy numbers, additional genetic and epigenetic modifications, and significant clinical differences are limited. In spite of these differences, distinctive clinical features of dup(15) syndrome, including early central hypotonia, developmental delay, intellectual disability, epilepsy, and autistic behavior, were defined. Battaglia's summary of clinical findings (2008) suggests that (a) in more than 75% of individuals with dup(15) hypotonia and lax ligaments, developmental delay and intellectual disability, autistic behavior, epilepsy, and minor dysmorphic features, involving mainly the face, are present; (b) from 25% to 50% of subjects are affected with brain abnormalities, growth retardation, and gastro-urinary tract defects; and (c) in less than 25% of subjects, microcephaly and congenital heart defects are observed. Wolpert *et al.* (2000a) documented that multiple maternal copies of the proximal 15q region may lead to one form of autistic disorder involving genes in the 15q11q13 region. Social, communicative, and behavioral function, and many clinical features are similar among individuals with autism associated with dup(15) and those with autism arising from other causes. The authors' review shows a much greater occurrence rate of seizures in dup(15) (69%) than reported in idiopathic autism (33%) (Tuchman and Rapin, 2002; Tuchman *et al*., 2009), a common delay in achieving motor milestones (77%), and hypotonia (77%) in dup(15) and a much lower occurrence rate of these abnormalities in idiopathic autism. Almost all individuals with inv dup(15) are affected with moderate to profound developmental delay and intellectual disability (Battaglia *et al*., 1997; Battaglia, 2008;

Crolla *et al*., 1995; Gillberg *et al*., 1991; Webb, 1994; Webb *et al*., 1998; Robinson *et al*., 1993;
Wolpert *et al*., 2000a).

Earlier studies of smaller groups of patients based on non-standardized criteria revealed autism
in 33% (Leana-Cox *et al*., 1994) and 36% (Crolla *et al*., 1995) of individuals diagnosed with
syndrome. Application of standardized assessment of autistic symptoms to a cohort of 29 children and
adults with dup(15) revealed that 69% of these subjects fulfilled the criteria of autism diagnosis
(Rineer *et al*., 1998).

Neuropathology of autism with dup(15) and with idiopathic autism. The current
knowledge of brain developmental alterations is based on results of examination of brains of nine
individuals diagnosed with dup(15), including seven subjects (78%) diagnosed with autism (Wegiel *et
al.*, 2012a,b). These studies demonstrate several striking differences and some similarities between
subjects diagnosed with autism associated with dup(15) and idiopathic autism. These patterns indicate
that autistic phenotype might be a product of etiologically, qualitatively, and quantitatively different
processes.

**Increased prevalence of brain transient overgrowth and macrocephaly in idiopathic
autism and microcephaly in dup(15) autism**. Macrocephaly, defined as head circumference greater
than the 97th percentile of the normal population, has been reported in more than 20% of autistic
subjects (Bailey *et al*., 1995; Bolton *et al*., 1994; Fombonne *et al*., 1999; Lainhart *et al*., 1997).
Increased brain weight was also reported in autism in postmortem studies (Bailey *et al*., 1993; Bauman
and Kemper, 1985). A short period of increased brain size starting at the age of less than 1 year
(Courchesne *et al*., 2003; Lainhart *et al*., 1997; Redcay and Courchesne, 2005) results in macrocephaly
in 37% of autistic children under the age of 4 years (Courchesne *et al*., 2001) and macrocephaly in
42% of the 19 twins diagnosed with idiopathic autism under the age of 16 years (Bailey *et al*., 1995).
Brain overgrowth is associated with increased number of neurons (Courchesne *et al*., 2011). However,
approximately 15% of 2- to 16-year-old autistic children are affected by microcephaly (Fombone *et al*.,
1999), which is associated frequently with a more severe clinical phenotype (Guerin *et al*., 1996; Hof
et al., 1991), including ID and other disorders (Fombone *et al*., 1999).

The characteristics of individuals with dup(15) are few, but published reports show opposite
proportions between macrocephalic and microcephalic subjects, unlike in idiopathic autism. In the

largest examined cohort (n = 107) with dup(15), macrocephaly was detected in only 2.8%, and
microcephaly in six times (16.8%) more subjects (Schroer *et al.*, 1998). The first postmortem study of
the brains of nine subjects diagnosed with dup(15), including seven subjects diagnosed with autism
(78%) revealed mean brain weight 303 g less than in the idiopathic autism group (p < 0.001) (Wegiel
et al 2012 a,b). Age-adjusted mean brain weight for dup(15) (n = 9), idiopathic autism (n = 10) and
control (n = 7) subjects was 1,171g, 1474 g, and 1378 g, respectively. The difference between the
dup(15) and control group was non-significant but suggestive (p = 0.06) (Fig. 1).

Two postmortem studies of idiopathic autism cohorts revealed epilepsy in six per 13 subjects
(46%; Wegiel *et al.*, 2010) and 3/10 (30%), whereas in the dup(15) group, epilepsy was diagnosed in
seven of nine cases (78%) (Wegiel *et al.*, 2012a). In the dup(15) cohort, sudden and unexplained death
in patients with epilepsy (SUDEP) was diagnosed in six of nine subjects (67%), and seizure-related
death was determined in one case (10%), resulting in 77% of cases of sudden death (Wegiel *et al.*,
2012a). Sudden death in the idiopathic autism cohort was reported in four of 13 subjects (31%, Wegiel
et al., 2010). These data suggest that the microcephaly and very early onset of seizures are risk factors
for SUDEP in the subpopulation of dup(15) subjects, and that this risk is at least two times greater in
dup(15) than in idiopathic autism.

**Neuropathological stratification of developmental abnormalities in dup(15) and idiopathic
autism cohorts**. Applications of an extended neuropathological protocol based on examination of
approximately 120 serial hemispheric sections per brain resulted in the detection of each
developmental abnormality larger than 2–3 mm. Three major types of developmental changes were
detected, including heterotopias, dysplastic changes, and abnormal neuronal proliferation. They were
found in all nine dup(15) and all 10 subjects with idiopathic autism; however, the type, topography,
and number of abnormalities show significant differences between these two cohorts (Fig. 2).

Heterotopias. Defects of migration resulting in heterotopias in the alveus, CA4, and dentate
gyrus (DG) occur very often in the dup(15) group (89%), are rare in individuals with idiopathic autism
(10%, p = 0.001), and are not present in control subjects. However, the prevalence of the heterotopias
in cerebellar white matter is comparable in dup(15) (56%) and idiopathic autism (60%). The
heterotopias in cerebral white matter are rare in both cohorts (11% and 10%, respectively). These three
patterns illustrate not only striking topographical differences in the distribution of defects of neuronal
migration but also significant differences between idiopathic autism and autism associated with
dup(15).

Dysplasia. Microdysgenesis resulting in focal developmental alterations of cytoarchitecture is also topographically selective and is detected mainly in the DG and cornu Ammonis in the hippocampal formation, and in the cerebral and cerebellar cortex. Dysplastic changes in the DG have been identified as hyperconvolution of the DG, duplication of the granular layer, massive protrusions of the granular layer into the molecular layer, focal thickening, thinning and fragmentation of the granular layer. The prevalence of dysplasia in the DG is several times higher (89%) in subjects with dup(15) than in the idiopathic autism group (10%; $p < 0.001$) (Fig. 3). In the DG, usually only one type of these changes is observed in idiopathic autism, whereas from two to five types are observed in each brain in dup(15) autism. However, dysplastic changes in the cornu Ammonis are rare in both cohorts, and the percentage of affected subjects is comparable: 20% in dup(15) autism and 22% in idiopathic autism.

Another feature distinguishing these two cohorts is cerebral cortex dysplasia, which is detected in 50% of subjects with dup(15) but is absent in idiopathic autism and in control subjects. The diversity of neocortical dysplastic changes, including multifocal cortical dysplasia with focal hypo- or acellularity, loss of vertical and horizontal organization, focal polymicrogyria and bottom-of-a-sulcus dysplasia, suggests that cortical abnormalities in dup(15) autism are the product of the distortion of several different mechanisms of cortex development.

The subependymal nodular dysplasia detected in the lateral ventricle in the occipital lobe in the brain of 15- and 39-year-old females diagnosed with dup(15) autism, and in 7- and 32-year-old males diagnosed with idiopathic autism are evidence of abnormal neuronal proliferation in some autistic subjects regardless of autism etiology and identify the predilection site for this developmental defect, which is detectable also on MRI scans (Wegiel *et al*., 2010, 2012a).

Causative link between developmental neuropathological changes, epilepsy, and sudden death in childhood. Similar hippocampal developmental abnormalities observed in sudden unexpected and unexplained death in childhood (SUDC) cases (Kinney *et al*., 2007) are considered an epileptogenic focus that might be triggered by infection, fever, or head trauma and result in seizures and unwitnessed death (Blum *et al*., 2000; Frysinger and Harper, 1990; Yang *et al*., 2001). SUDC in two subjects and SUDEP in five cases with dup(15) and autism and several-fold higher prevalence of hippocampal and cortical dysplasia than in idiopathic autism appears to be the clinicopathological criterion for stratification between and within these cohorts. These developmental alterations are not pathognomonic of an "epileptic brain" (Kinney *et al*., 2007), but the combination of microcephaly, the

2.5-fold higher prevalence of several types of developmental abnormalities, the 2.3-fold higher prevalence of epilepsy, and the six-times higher prevalence of epilepsy-related death in the dup(15) cohort suggests that unique genetic and molecular modifications and developmental structural defects distinguish these two cohorts of autistic subjects.

The link between dysplastic changes in the cerebellar flocculus and atypical gaze. Cerebellar abnormalities are among the most consistent developmental alterations detected in autism (Bauman and Kemper, 1996; Courchesne *et al*., 2001; Kemper and Bauman, 1993; Ritvo *et al*., 1986; Whitney *et al*., 2008, 2009). A reduced number of Purkinje cells (PCs) has been detected in 72% of the reported autism cases (Palmen *et al*. review, 2004), but studies by Whitney *et al*. suggest that the reduced number of PCs is not the effect of developmental deficit, but is instead t he result of early neuronal loss (Whitney *et al*., 2008, 2009). The prevalence of defective migration of cortical neurons and dentate nucleus neurons in the cerebellar white matter was almost identical in dup(15) autism and idiopathic autism (56% and 60%, respectively). Four types of cerebellar dysplasia, including nodulus, flocculus, and vermis dysplasia, and focal polymicrogyria, were found in dup(15) autism and idiopathic autism. Vermis dysplasia and focal polymicrogyria were rare in both groups.

However, a portion of the flocculus and a small portion of the nodulus, developmentally related to the flocculus (flocculus-like" region of the ventral paraflocculus; Tan *et al*., 1995), were affected by dysplastic changes. Flocculus dysplasia is associated with a striking deficit of PCs and unipolar brush cells, an almost complete lack of inhibitory basket and stellate cells in the molecular layer, and reduction of the number of granule cells. This abnormal flocculus cytoarchitecture appears to be an indicator of the profound disruption of the olivo-floccular circuitry and severe functional alterations. The flocculus participates in the control of eye motion (Leung *et al*., 2000), and coordination of eye and head movements during active gaze shifts by modulating the vestibulo-ocular reflex (Belton and McCrea, 1999). Dysplastic changes are observed in the nodulus of autistic and control subjects, but the function of the affected region of the nodulus is not clear. The presence of dysplasia in the flocculus in 75% of individuals with dup(15) autism (Wegiel *et al*., 2012b), in 50% and 67% of idiopathic autism cases (Wegiel *et al*., 2012a,c), and 20% of control subjects (Wegiel *et al*., 2012c) and the presence of olivary dysplasia in three of five autistic subjects and ectopic neurons related to the olivary complex in two cases reported by Bailey *et al*. (1998) indicate that both major structural and functional components of the olivo-floccular circuitry are prone to developmental defects, most likely contributing to the atypical gaze of autistic subjects. These findings also suggest that flocculus

developmental defects are observed in autistic subjects regardless of etiology. Individuals diagnosed with idiopathic autism and dup(15) autism reveal altered oculomotor functions, including atypical gaze, impairments in smooth pursuit, and deficits in facial perceptions, suggesting defects in the olivo-floccular neuronal circuit. These defects are reported early in the development of children with autism (Mundy *et al*., 1986; Dawson *et al*., 1998) and contribute to deficits in using gaze to understand the intentions of other people and their mental states (Baron-Cohen, 1995; Baron-Cohen *et al*., 1999, 2001; Leekam *et al*., 1998, 2000).

Increased levels of secreted amyloid precursor protein-alpha (sAPP) and reduced levels of Aβ40 and Aβ42 in the blood plasma. Significantly lower concentrations of both $A\beta_{1-40}$ and $A\beta_{1-42}$, and a reduced ratio of $A\beta_{40/42}$ detected in the blood plasma of 52 autistic children 3 to 16 years of age compared to 39 age-matched control subjects were attributed to the loss of Aβ equilibrium between the brain and blood (Al-Ayadhi *et al*., 2012). Significantly increased levels of sAPP-α in blood plasma in 60% of autistic children (Sokol *et al.*, 2006) indicate enhanced non-amyloidogenic APP processing by α-secretase. Higher levels of sAPP-α in blood plasma were especially prominent in autistic subjects with aggressive behavior (Ray *et al*., 2011; Sokol *et al*., 2006). Enhanced non-amyloidogenic cleavage of APP with α-secretase is associated not only with autism (Bailey *et al*., 2008; Ray *et al*., 2011; Sokol *et al*., 2006; Sokol *et al*., 2011; Wegiel *et al*., 2012b), but also with FXS (Sokol *et al*., 2011; Westmark and Malter, 2007; Westmark *et al*., 2011). Due to the neurotrophic activity of sAPP-α, increased levels of this APP metabolite are considered a co-factor contributing to brain overgrowth in autism and FXS (Sokol *et al*., 2011). The fragile X mental retardation protein (FMRP) binds to and represses translation of APP mRNA. The absence of FMRP in people diagnosed with FXS results in upregulation of APP, $A\beta_{40}$, and $A\beta_{42}$. Similar upregulation is detected in *Fmr1^{KO}* mice (Westmark and Malter, 2007); however, genetic reduction of *AβPP* by removal of one *App* allele in *Fmr1^{KO}* mice reverses the FXS phenotype and increases blood plasma levels of $A\beta_{1-42}$ to control levels (Westmark *et al*., 2011).

Enhanced accumulation of amino-terminally truncated Aβ in neuronal cytoplasm. Neuronal proteolytic cleavage of APP by β- and γ-secretases (amyloidogenic pathway) results in release of $A\beta_{1-40}$ and $A\beta_{1-42}$, which are able to form fibrillar deposits in the extracellular space (amyloid plaques) and in the wall of brain vessels (amyloid angiopathy). $A\beta_{17-40}$ and $A\beta_{17-42}$ is a product of α- and γ-secretases (p3 peptide) in the non-amyloidogenic pathway (Iversen *et al*. 1995; Selkoe, 2001). Aβ is generated and detected in the endoplasmic reticulum/Golgi apparatus and endosomal-lysosomal pathway (Cook D.G. *et al*., 1997; Glabe, 2001; Greenfield *et al*., 1999; Hartmann *et al*., 1997; Wilson *et al*., 1999),

multivesicular bodies (Takahashi *et al*., 2002), and mitochondria (Bayer and Wirths, 2010; Casperson *et al*., 2005). Aβ peptides differ in oligomerization and fibrillization as well as toxicity. Intraneuronal accumulation has been reported in normal human brain (Wegiel *et al*., 2007). Enhanced accumulation has been proposed as an early alteration in Alzheimer's disease (AD) and in transgenic mouse models of AD (Bayer and Wirths, 2010; Gouras *et al*., 2010; Gyure *et al*., 2001; Mochizuki *et al*., 2000; Winton *et al*., 2011).

Intraneuronal Aβ in human brain is mainly amino-terminally truncated $A\beta_{17-40}$ and $A\beta_{17-42}$ (Wegiel *et al*., 2007). Cytoplasmic Aβ in neurons is a reflection of the balance between its rate of synthesis, accumulation in cytoplasmic organelles, and degradation. The extracellular level of Aβ is a reflection of neuronal production and extracellular oligomerization, fibrilization, deposition, and disposal, including drawing of the excessive amounts through the blood-brain barrier to the blood (Weller *et al*., 1998). The morphology and amount of intracellular deposits of Aβ are neuron-type–specific and show a broad spectrum of differences in developing and aging brains and in brains affected by pathology. Increased level of sAPP-α in the blood plasma of autistic subjects is linked to enhanced intraneuronal accumulation of amino-terminally truncated $A\beta_{17-40/42}$ in the neurons of autistic subjects (Wegiel *et al*., 2012b).

Stratification of Aβ accumulation in neurons in the dup(15) autism and idiopathic autism. Postmortem study of 12 brain structures and neuronal populations (frontal, temporal, and occipital cortex; amygdala, thalamus, lateral geniculate body, DG; CA1 and CA4 sectors and dentate nucleus in the hippocampal formation; and PCs) revealed higher Aβ load in neurons in 11 subregions in dup(15) autism than in idiopathic autism ($p < 0.0001$) and in control subjects ($p < 0.0001$). In eight regions, cytoplasmic Aβ load was significantly higher in idiopathic autism than in control subjects ($p < 0.001$).

Excessive accumulation of Aβ in two autistic cohorts was neuron-type–specific. Classification of neuronal Aβ immunoreactivity as strong, moderate, and weak revealed two types of alterations. Type 1 is characterized by a significant increase in the percentage of neurons with strong Aβ immune-reactivity, defined as a condensed mass of indistinguishable small and large immunoreactive granules occupying a large portion of neuron perikaryon. Type 1 of Aβ accumulation is typical for the amygdala, thalamus, and lateral geniculate body (LGB) (Fig. 4). Stratification of dup(15)autism and idiopathic autism cohorts is reflected in a 7.6-fold increase of the percentage of strongly positive neurons in the amygdala and thalamus and a 4.5-fold increase in the LGB in dup(15) autism in comparison to the control cohort. In idiopathic autism, the increase was 5.3x, 6.3x, and 3.9x,

respectively, in comparison to in the control subjects. Type 2 of Aβ intraneuronal accumulation is distinguished by a relatively low percentage of neurons with strong Aβ immunoreactivity, but a higher total percentage of Aβ-positive neurons. This pattern was typical for pyramidal neurons in all three examined neocortical regions (frontal, temporal, and occipital cortex), and the total percentage of Aβ-positive neurons was higher in dup(15) than in the autism and control groups ($p < 0.001$).

$Aβ_{1-40}$ and $Aβ_{1-42}$ in diffuse plaques in autism. Diffuse amorphous nonfibrillar Aβ deposits are classified as preplaques (Mann *et al.*, 1989) or pre-amyloid deposits (Tagliavini *et al.*, 1989); however, diffuse deposits in human cerebellar cortex and the parvocellular layer of the presubiculum do not fibrilize, regardless of age or stage of AD (Wisniewski *et al.*, 1998). Diffuse plaques were found in two of the 11 subjects diagnosed with ASD (51 and 52 years old) and in one of nine subjects diagnosed with dup(15) and autism (Fig. 5). These subjects were the oldest in both examined groups. Plaques were nonfibrillar, thioflavin S–negative, but in contrast to amino-terminally truncated $Aβ_{17-40/42}$ in neurons, they contained full-length $Aβ_{1-40/42}$ (Wegiel *et al.*, 2012b). Diffuse plaques are also observed in young people diagnosed with DS, but they are $Aβ_{17-40/42}$-positive (Gowing *et al.*, 1994;, Lalowski *et al.*, 1996).

Both in the plaque perimeter and in plaque-free areas, numerous astrocytes and some microglial cells contain amyloid, but only amino-terminally truncated $Aβ_{17-40/42}$. Focal enhanced proliferation of astrocytes, accumulation of Aβ in their cytoplasm, and accelerated death results in Aβ deposition, mainly in the perivascular space.

These findings suggest that the pattern of metabolic alterations of APP processing and Aβ accumulation is comparable in two autistic cohorts, but the severity of metabolic changes is significantly intensified in dup(15) autism in comparison to idiopathic autism. Enhanced APP processing with α- and γ-secretase increases the percentage of Aß-positive neurons and intracellular amyloid load in dup(15) autism with microcephaly. Increased levels of blood plasma neurotrophic sAPP-α detected in 60% of subjects with idiopathic autism justified the hypothesis that the increased level of the product of α-secretase may help identify a subset of children in which early brain overgrowth is sufficient for development of autism and might be a marker of the mechanism that regulates brain overgrowth (Sokol *et al.*, 2011). One may hypothesize that in subjects with dup(15), genetic factors contribute to microcephaly and dominate over metabolic modifications and elevated levels of neurotrophic products of APP processing.

Closing remarks. Comparative postmortem studies of the brains of individuals diagnosed with dup(15) autism identify a cluster of neuropathological findings differentiating this cohort of autistic subjects with genetically identified autism etiology from a cohort of subjects with idiopathic autism. These studies support the recommendation by Happe *et al*. (2006) for fractionation of autism into "autisms" with different etiologies, clinical presentations, and neuropathology, and most likely requiring different preventive strategies and different treatments.

Because the complex nature of developmental abnormalities increases the risk of death in childhood and early adulthood in the dup(15) autism cohort, postmortem study appears to reflect both developmental abnormalities associated with this genetic trait, and a particular combination of factors contributing to early death. The list of factors defining the risk of early death and the detected pattern of neuropathological changes includes: (a) maternal origin dup(15), (b) autism, (c) more severe clinical phenotypes with ID, early-onset and severe or intractable seizures, and increased prevalence of SUDEP, (d) high prevalence of microcephaly, (e) several-fold increase in the number of developmental abnormalities, including defects of migration and multifocal defects of cytoarchitecture, especially numerous in the hippocampal formation, and (f) several-fold increase in the percentage of neurons with increased amyloid load, reflecting enhanced APP processing in non-amyloidogenic pathway with α- and γ-secretase ($A\beta_{17-40/42}$); enhanced proliferation and activation of $A\beta_{17-40/42}$-positive astrocytes, enhanced rate of astrocytes death, and in some cases, an early onset of diffuse plaques containing $A\beta_{1-40/42}$.

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Figure legend

Figure 1. Significant reduction of mean brain weight (g) in the dup(15) autism group (1,171 g) in comparison to the idiopathic autism (iA) cohort (1,474 g; $p < 0.001$), and insignificant but suggestive reduction in comparison to the control (C) group (1,378 g; $p < 0.06$).

Figure 2. The mean number of developmental alterations detected in postmortem evaluation of serial hemispheric sections was 2.5x higher in the dup(15) autism group (7.1/case) than in the idiopathic autism (iA) group (2.8/case).

Figure 3. Topography and morphology of 11 types of developmental abnormalities in the brain of a 24-year–old male diagnosed with dup(15), autism, severe seizures, and SUDEP: dentate gyrus (DG) hyperconvolution and DG heterotopia in the CA4 (a); DG granule cell layer protrusion (b; arrowhead), duplication (c), focal thinning and discontinuity (d), granule cell layer fragmentation (e; arrowhead); multifocal microdysgenesis within CA4 (e, two arrowheads); larger magnification of different types of microdysgenesis within CA4 (f, g); cerebellar heterotopia with morphology of cerebellar deep nuclei (h, i) and with modified morphology of cerebellar cortex (j, k); and dysplasia in the cerebellar nodulus (l) and flocculus (m).

Figure 4. The percentage of neurons with very high intracellular Aβ load is significantly higher in the amygdala, thalamus, and Purkinje cells in dup(15) autism than idiopathic autism and control subjects ($p < 0.0001$). In the frontal, temporal, and occipital cortex, the total percentage of Aβ-positive neurons (neurons with strong, moderate, and weak Aβ immunoreactivity) is significantly higher in dup(15) autism than in idiopathic autism and control groups ($p < 0.0001$).
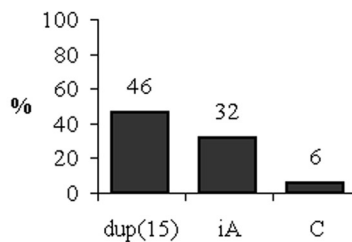
Figure 5. In the oldest subject examined postmortem in the dup(15) group (39-year-old autistic female), and two subjects with autistic disorder of unknown etiology (51 and 52 years old), numerous diffuse $Aβ_{1-40/42}$-positive plaques were detected in the cortical ribbon (a, b, c, respectively).
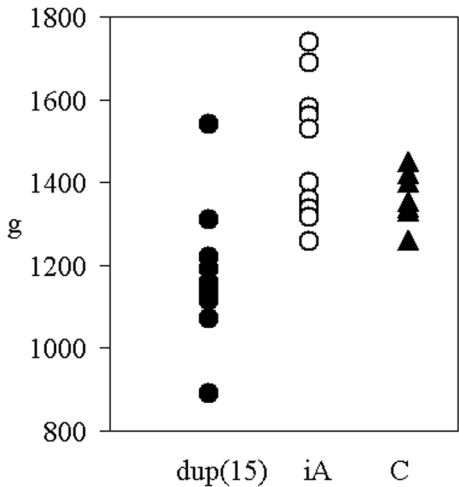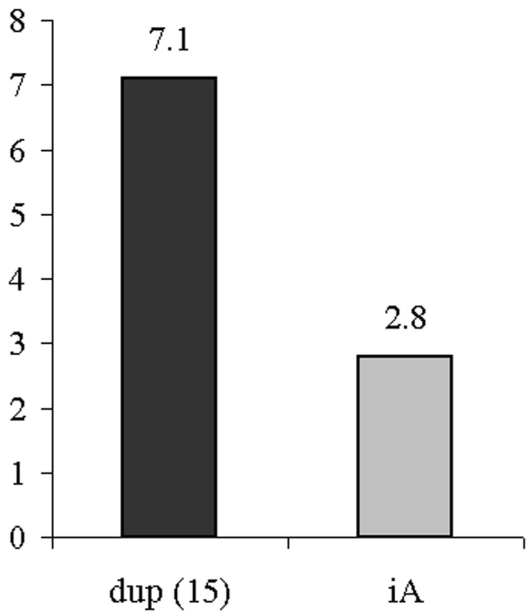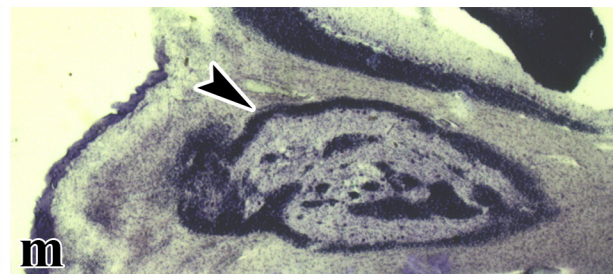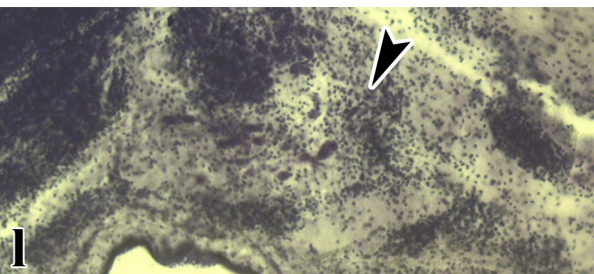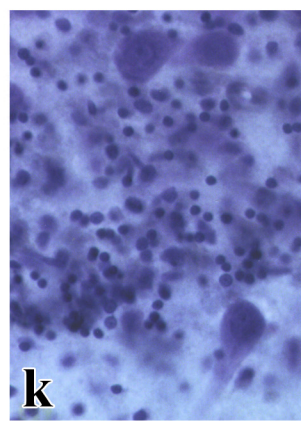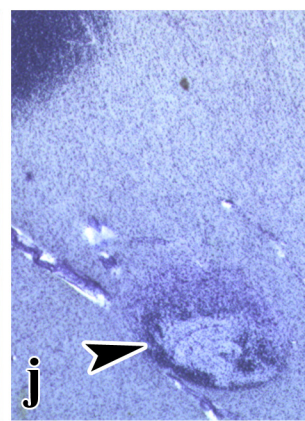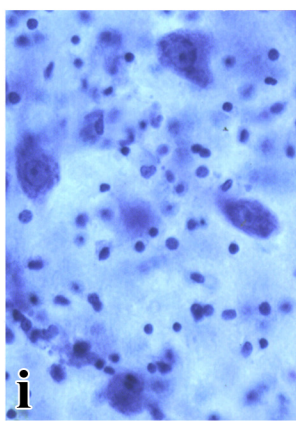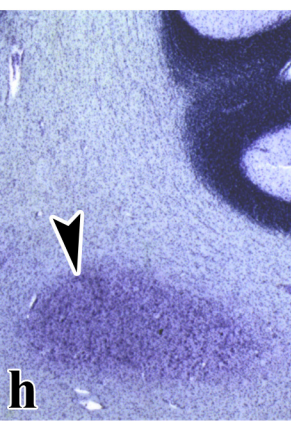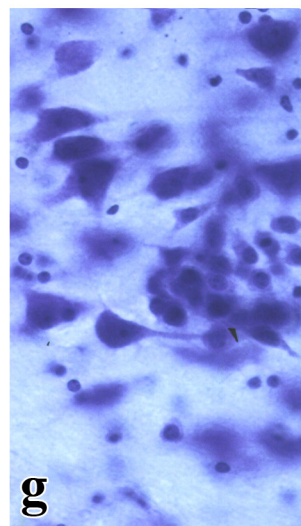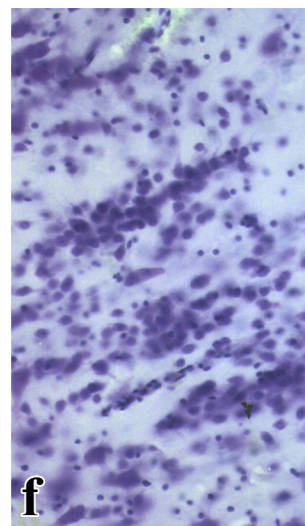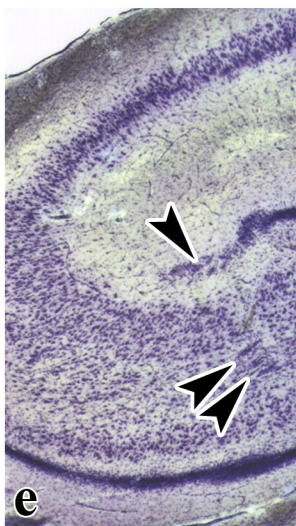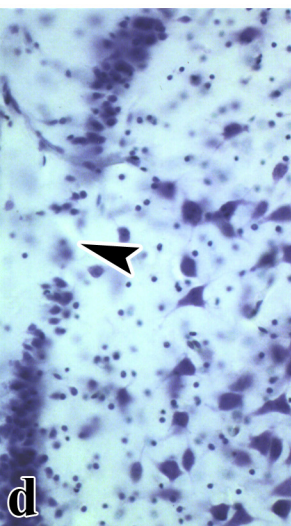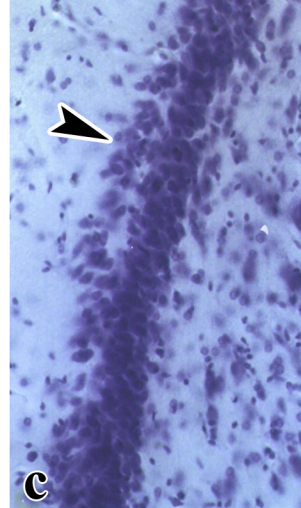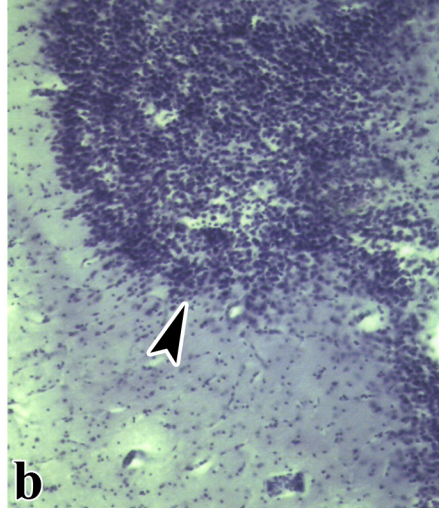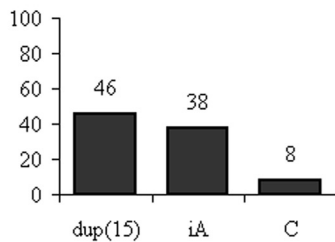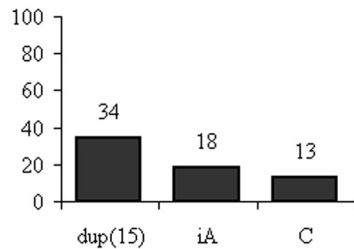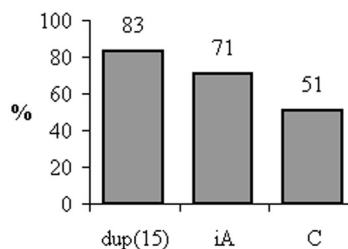
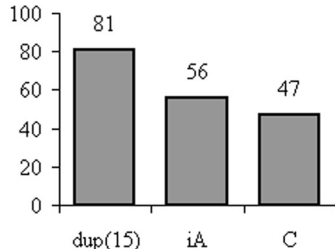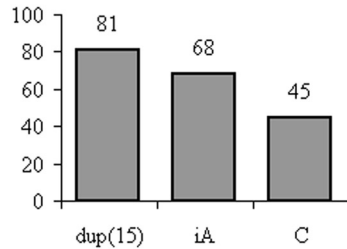|		
Amygdala	**Thalamus**	**Purkinje Cells**
Frontal C.	**Temporal C.**	**Occipital C.**